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Chiang et al.

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(54) **INACTIVATED CANINE INFLUENZA VACCINES AND METHODS OF MAKING AND USES THEREOF**

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(51) **Int. Cl.**

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(52) **U.S. Cl.**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to canine influenza virus strains, and vaccines and compositions. The present invention also relates to reagents and methods allowing their detection, methods of vaccination as well as methods of producing these reagents and vaccines.

13 Claims, 21 Drawing Sheets

Specification includes a Sequence Listing.

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Figure 1A

SEQ ID NO:	Type	Gene Description
1	DNA	DNA encoding HA protein from #4 isolate (in eggs) of H3N2 CIV
2	Protein	HA protein from #4 isolate (in eggs) of H3N2 CIV
3	DNA	DNA encoding HA protein from #4 isolate (in MDCK cells) of H3N2 CIV
4	Protein	HA protein from #4 isolate (in MDCK cells) of H3N2 CIV
5	DNA	DNA encoding HA protein from #8 isolate (in eggs) of H3N2 CIV
6	Protein	HA protein from #8 isolate (in eggs) of H3N2 CIV
7	DNA	DNA encoding NA protein from #4 isolate (in eggs) of H3N2 CIV
8	Protein	NA protein from #4 isolate (in eggs) of H3N2 CIV
9	DNA	DNA encoding NA protein from #4 isolate (in MDCK cells) of H3N2 CIV
10	Protein	NA protein from #4 isolate (in MDCK cells) of H3N2 CIV
11	DNA	DNA encoding NA protein from #8 isolate (in eggs) of H3N2 CIV
12	Protein	NA protein from #8 isolate (in eggs) of H3N2 CIV
13	oligo	H3N2 NA FWD: 5'-GGG ACC ACG CTG AAC AAT AA-3'
14	oligo	H3N2 NA REV: 5'-TGA AAC GGA ACA CCC AAC TC -3'
15	oligo	H3N8 NA FWD: 5'-GTT CGC CCT CAG AAT GTA GAA-3'
16	oligo	H3N8 NA REV: 5'-CCT ATA CGG ACT TCG ATC CTT TAT T-3'
17	oligo	Ca.H3N2.HA.390R
18	oligo	Ca.H3N2.HA.259F
19	oligo	Ca.H3N2.HA.743F
20	oligo	Ca.H3N2.HA.1276F
21	oligo	Ca.H3N2.HA.1569F
22	oligo	Ca.H3N2.HA.871R
23	oligo	Ca.H3N2.HA.1408R
24	oligo	Ca.H3N2.NA.429F
25	oligo	Ca.H3N2.NA.484R
26	oligo	Ca.H3N2.NA.975F
27	oligo	Ca.H3N2.NA.1023R
28	oligo	Ca.H3N2.1229F
29	oligo	Ca.H3N2.1279R

Figure 1B

SEQ ID NO:	type	Gene Description
30	DNA	DNA encoding HA protein of H3N8 CIV strain A/Ca/CT/85863/11 (GenBank KM359807.1)
31	protein	HA protein of H3N8 CIV strain A/Ca/CT/85863/11 (GenBank AIN25426.1)
32	DNA	DNA encoding NA protein of H3N8 CIV strain A/Ca/CT/85863/11
33	protein	NA protein of H3N8 CIV strain A/Ca/CT/85863/11
34	DNA	DNA encoding HA protein of H3N8 CIV strain A/canine/NY/120106.2/2011 (GenBank KM359803.1)
35	protein	HA protein of H3N8 CIV strain A/canine/NY/120106.2/2011 (GenBank AIN25422.1)
36	DNA	DNA encoding NA protein of H3N8 CIV strain A/canine/NY/120106.2/2011 (GenBank KM359831.1)
37	protein	NA protein of H3N8 CIV strain A/canine/NY/120106.2/2011 (GenBank AIN25464.1)
38	DNA	DNA encoding HA protein of H3N8 CIV strain WY/86033/07
39	protein	HA protein of H3N8 CIV strain WY/86033/07
40	DNA	DNA encoding NA protein of H3N8 CIV strain WY/86033/07
41	protein	NA protein of H3N8 CIV strain WY/86033/07
42	DNA	DNA encoding NA protein (SEQ ID NO:37) of H3N8 CIV strain A/canine/NY/120106

Figure 2

Real-time RT-PCR genotyping of CIVs

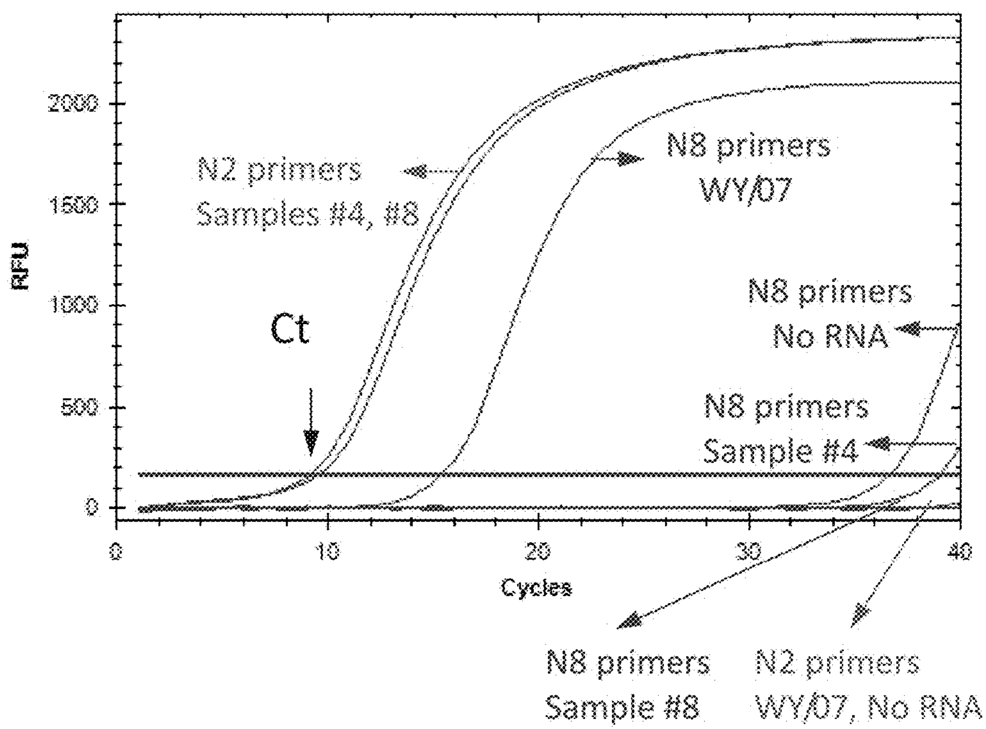
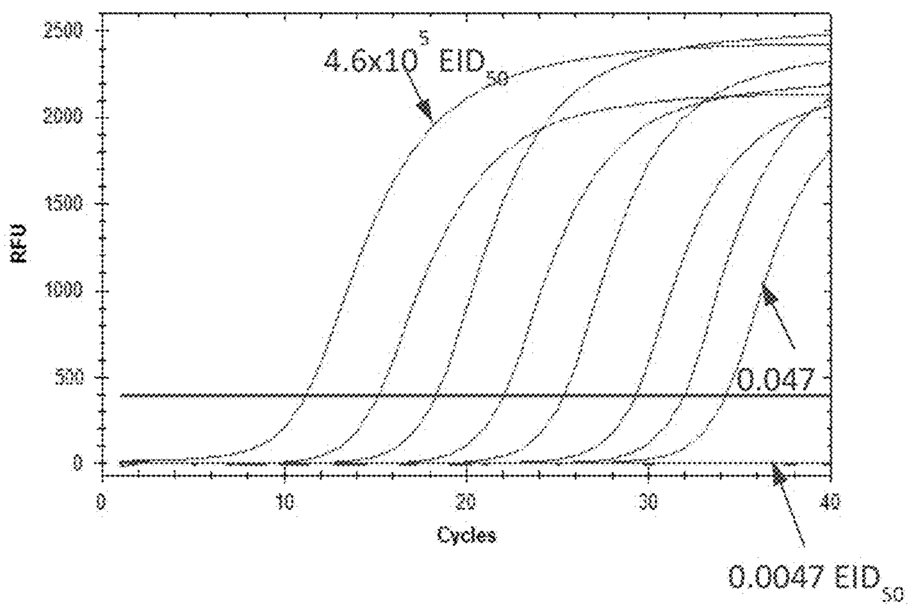


Figure 3

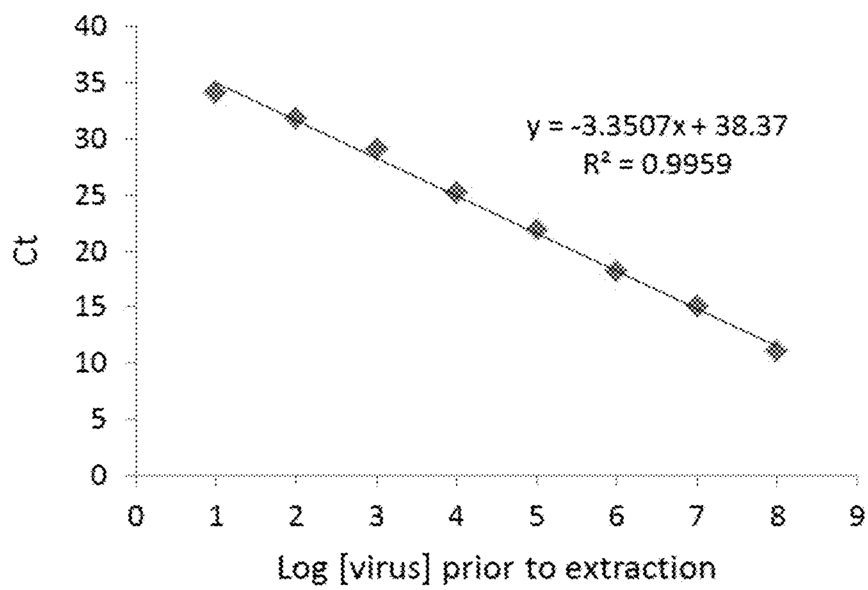
Sensitivity of the subtyping assay



Detection limit = 0.047 EID_{50} units

Figure 4

Efficiency of the subtyping assay



Amplification factor = 1.99
Efficiency = 98.81

Figure 5A

Protein sequence alignment of HA protein of H3N2 and sequence identity

```
                       1                                                50
SEQ ID NO:2     (1)    MKTVIALSYIFCLAFGQNLLGNENNAATLCLGHHAVPNGTMVKTITDDQI
SEQ ID NO:4     (1)    MKTVIALSYIFCLAFGQNLLGNENNAATLCLGHHAVPNGTMVKTITDDQI
SEQ ID NO:6     (1)    MKTVIALSYIFCLAFGQNLLGNENNAATLCLGHHAVPNGTMVKTITDDQI
                       51                                              100
SEQ ID NO:2    (51)    EVTNATELVQNSSTGKICNNPHKILDGRDCTLIDALLGDPHCDVFQNETW
SEQ ID NO:4    (51)    EVTNATELVQNSSTGKICNNPHKILDGRDCTLIDALLGDPHCDVFQNETW
SEQ ID NO:6    (51)    EVTNATELVQNPSTGKICNNPHKILDGRDCTLIDALLGDPHCDVFQNETW
                       101                                             150
SEQ ID NO:2   (101)    DLFVERSNAFSNCYPYDVPDYASLRSIVASSGTLEFITEGFTWAGVTQNG
SEQ ID NO:4   (101)    DLFVERSNAFSNCYPYDVPDYASLRSIVASSGTLEFITEGFTWAGVTQNG
SEQ ID NO:6   (101)    DLFVERSNAFSNCYPYDVPDYASLRSIVASSGTLEFITEGFTWAGVTQNG
                       151                                             200
SEQ ID NO:2   (151)    GSGACKRGPANSFFSRLNWLTKSGNTYPVLNVTMPNNNNFDKLYIWGVHH
SEQ ID NO:4   (151)    GSGACKRGPANSFFSRLNWLTKSGNTYPVLNVTMPNNNNFDKLYIWGVHH
SEQ ID NO:6   (151)    GSGACKRGPANSFFSRLNWLTKSGNTYPVLNVTMPNNNNFDKLYIWGVHH
                       201                                             250
SEQ ID NO:2   (201)    PSTNQEQTSLYIQASGRVTVSTRRSQQTIIPNIGSRPLVRGQSGRISVYW
SEQ ID NO:4   (201)    PSTNQEQTSLYIQASGRVKVSTRRSQQTIIPNIGSRPLVRGQSGRISVYW
SEQ ID NO:6   (201)    PSTNQEQTSLYIQASGRVTVSTRRSQQTIIPNIGSRPLVRGQSGRISVYW
                       251                                             300
SEQ ID NO:2   (251)    TIVKPGDILVINSNGNLIAPRGYFKMHIGKSSIMRSDAPIDTCISECITP
SEQ ID NO:4   (251)    TIVKPGDILVINSNGNLIAPRGYFKMHIGKSSIMRSDAPIDTCISECITP
SEQ ID NO:6   (251)    TIVKPGDILVINSNGNLIAPRGYFKMHIGKSSIMRSDAPIDTCISECITP
                       301                                             350
SEQ ID NO:2   (301)    NGSIPNEKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPERQTRGLFGA
SEQ ID NO:4   (301)    NGSIPNEKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPERQTRGLFGA
SEQ ID NO:6   (301)    NGSIPNEKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPERQTRGLFGA
                       351                                             400
SEQ ID NO:2   (351)    IAGFIENGWEGMVDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRV
SEQ ID NO:4   (351)    IAGFIENGWEGMVDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRV
SEQ ID NO:6   (351)    IAGFIENGWEGMVDGWYGFRHQNSEGTGQAADLKSTQAAIDQINGKLNRV
                       401                                             450
SEQ ID NO:2   (401)    IEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKVDLWSYNAELLVALENQ
SEQ ID NO:4   (401)    IEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKVDLWSYNAELLVALENQ
SEQ ID NO:6   (401)    IEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKVDLWSYNAELLVALENQ
                       451                                             500
SEQ ID NO:2   (451)    HTIDLTDSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNG
SEQ ID NO:4   (451)    HTIDLTDSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNG
SEQ ID NO:6   (451)    HTIDLTDSEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNG
                       501                                             550
SEQ ID NO:2   (501)    TYDHNIYRDEAVNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGF
SEQ ID NO:4   (501)    TYDHNIYRDEAVNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGF
SEQ ID NO:6   (501)    TYDHNIYRDEAVNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGF
                       551        566
SEQ ID NO:2   (551)    IMWACQRGNIRCNICI
SEQ ID NO:4   (551)    IMWACQRGNIRCNICI
SEQ ID NO:6   (551)    IMWACQRGNIRCNICI
```

```
Sequence identity:
SEQ ID NO:2 v. SEQ ID NO:4:     99.6%
SEQ ID NO:2 v. SEQ ID NO:6:     99.6%
SEQ ID NO:4 v. SEQ ID NO:6:     99.6%
```

Figure 5B

DNA sequence alignment of polynucleotide encoding HA protein of H3N2 and sequence identity

```
                    1                                                 50
SEQ ID NO:1   (1)   ATGAAAACTGTTATTGCTTTAAGCTATATTTTCTGCCTGGCTTTTGGTCA
SEQ ID NO:3   (1)   ATGAAAACTGTTATTGCTTTAAGCTATATTTTCTGCCTGGCTTTTGGTCA
SEQ ID NO:5   (1)   ATGAAAACTGTTATTGCTTTAAGCTATATTTTCTGCCTGGCTTTTGGTCA
                    51                                               100
SEQ ID NO:1   (51)  GAATCTTCTAGGAAATGAAAATAATGCTGCAACACTATGCCTGGGACATC
SEQ ID NO:3   (51)  GAATCTTCTAGGAAATGAAAATAATGCTGCAACACTATGCCTGGGACATC
SEQ ID NO:5   (51)  GAATCTTCTAGGAAATGAAAATAATGCTGCAACACTATGCCTGGGACATC
                    101                                              150
SEQ ID NO:1   (101) ATGCAGTGCCGAACGGGACAATGGTGAAAACTATCACAGACGATCAAATT
SEQ ID NO:3   (101) ATGCAGTGCCGAACGGGACAATGGTGAAAACTATCACAGACGATCAAATT
SEQ ID NO:5   (101) ATGCAGTGCCGAACGGGACAATGGTGAAAACTATCACAGACGATCAAATT
                    151                                              200
SEQ ID NO:1   (151) GAGGTGACCAACGCCACCGAGCTAGTCCAAAACTCCTCAACAGGGAAAAT
SEQ ID NO:3   (151) GAGGTGACCAACGCCACCGAGCTAGTCCAAAACTCCTCAACAGGGAAAAT
SEQ ID NO:5   (151) GAGGTGACCAACGCCACCGAGCTAGTCCAAAACCCCTCAACAGGGAAAAT
                    201                                              250
SEQ ID NO:1   (201) ATGCAACAATCCCCACAAGATTCTTGATGGGAGGGACTGCACACTAATAG
SEQ ID NO:3   (201) ATGCAACAATCCCCACAAGATTCTTGATGGGAGGGACTGCACACTAATAG
SEQ ID NO:5   (201) ATGCAACAATCCCCACAAGATTCTTGATGGGAGGGACTGCACACTAATAG
                    251                                              300
SEQ ID NO:1   (251) ATGCCCTACTAGGGGACCCACACTGTGACGTCTTCCAAAATGAGACATGG
SEQ ID NO:3   (251) ATGCCCTACTAGGGGACCCACACTGTGACGTCTTCCAAAATGAGACATGG
SEQ ID NO:5   (251) ATGCCCTACTAGGGGACCCACACTGTGACGTCTTCCAAAATGAGACATGG
                    301                                              350
SEQ ID NO:1   (301) GACCTTTTTGTGGAACGAAGCAATGCTTTTAGCAATTGTTACCCTTATGA
SEQ ID NO:3   (301) GACCTTTTTGTGGAACGAAGCAATGCTTTTAGCAATTGTTACCCTTATGA
SEQ ID NO:5   (301) GACCTTTTTGTGGAACGAAGCAATGCTTTTAGCAATTGTTACCCTTATGA
                    351                                              400
SEQ ID NO:1   (351) TGTACCAGACTATGCATCCCTCCGATCCATAGTTGCATCATCAGGCACAT
SEQ ID NO:3   (351) TGTACCAGACTATGCATCCCTCCGATCCATAGTTGCATCATCAGGCACAT
SEQ ID NO:5   (351) TGTACCAGACTATGCATCCCTCCGATCCATAGTTGCATCATCAGGCACAT
                    401                                              450
SEQ ID NO:1   (401) TGGAGTTCATCACTGAAGGTTTCACTTGGGCAGGAGTAACTCAAAATGGA
SEQ ID NO:3   (401) TGGAGTTCATCACTGAAGGTTTCACTTGGGCAGGAGTAACTCAAAATGGA
SEQ ID NO:5   (401) TGGAGTTCATCACTGAAGGTTTCACTTGGGCAGGAGTAACTCAAAATGGA
                    451                                              500
SEQ ID NO:1   (451) GGAAGCGGTGCTTGTAAAAGGGGACCTGCTAATAGTTTCTTCAGTAGATT
SEQ ID NO:3   (451) GGAAGCGGTGCTTGTAAAAGGGGACCTGCTAATAGTTTCTTCAGTAGATT
SEQ ID NO:5   (451) GGAAGCGGTGCTTGTAAAAGGGGACCTGCTAATAGTTTCTTCAGTAGATT
                    501                                              550
SEQ ID NO:1   (501) AAATTGGTTAACTAAATCAGGAAATACATATCCAGTGTTGAATGTGACTA
SEQ ID NO:3   (501) AAATTGGTTAACTAAATCAGGAAATACATATCCAGTGTTGAATGTGACTA
SEQ ID NO:5   (501) AAATTGGTTAACTAAATCAGGAAATACATATCCAGTGTTGAATGTGACTA
                    551                                              600
SEQ ID NO:1   (551) TGCCAAACAACAACAATTTCGACAAATTATACATTTGGGGAGTTCATCAC
SEQ ID NO:3   (551) TGCCAAACAACAACAATTTCGACAAATTATACATTTGGGGAGTTCATCAC
SEQ ID NO:5   (551) TGCCAAACAACAACAATTTCGACAAATTATACATTTGGGGAGTTCATCAC
                    601                                              650
SEQ ID NO:1   (601) CCAAGCACTAATCAAGAACAAACCAGCCTGTATATTCAGGCCTCAGGAAG
SEQ ID NO:3   (601) CCAAGCACTAATCAAGAACAAACCAGCCTGTATATTCAGGCCTCAGGAAG
SEQ ID NO:5   (601) CCAAGCACTAATCAAGAACAAACCAGCCTGTATATTCAGGCCTCAGGAAG
```

Figure 5C

		651 700
SEQ ID NO:1	(651)	AGTCAGAGTCTCTACCAGGAGAAGCCAACAGACCATAATCCCAAACATTG
SEQ ID NO:3	(651)	AGTCAAAGTCTCTACCAGGAGAAGCCAACAGACCATAATCCCAAACATTG
SEQ ID NO:5	(651)	AGTCAGAGTCTCTACCAGGAGAAGCCAACAGACCATAATCCCAAACATTG
		701 750
SEQ ID NO:1	(701)	GATCTAGACCCTTGGTAAGGGCCCAATCTGGCAGAATAAGCGTACATTGG
SEQ ID NO:3	(701)	GATCTAGACCCTTGGTAAGGGCCCAATCTGGCAGAATAAGCGTATATTGG
SEQ ID NO:5	(701)	GATCTAGACCCTTGGTAAGGGCCCAATCTGGCAGAATAAGCGTATATTGG
		751 800
SEQ ID NO:1	(751)	ACAATAGTCAAACCTGGAGACATACTGGTAATAAACAGTAATGGAAACCT
SEQ ID NO:3	(751)	ACAATAGTCAAACCTGGAGACATACTGGTAATAAACAGTAATGGAAACCT
SEQ ID NO:5	(751)	ACAATAGTCAAACCTGGAGACATACTGGTAATAAACAGTAATGGAAACCT
		801 850
SEQ ID NO:1	(801)	AATCGCTCCTCGAGGATACTTCAAAATGCACATTGGGAAAAGCTCAATAA
SEQ ID NO:3	(801)	AATCGCTCCTCGAGGATACTTCAAAATGCACATTGGGAAAAGCTCAATAA
SEQ ID NO:5	(801)	AATCGCTCCTCGAGGATACTTCAAAATGCACATTGGGAAAAGCTCAATAA
		851 900
SEQ ID NO:1	(851)	TGAGATCAGATGCACCTATTGACACCTGCATTTCCGAATGTATCACCCCG
SEQ ID NO:3	(851)	TGAGATCAGATGCACCTATTGACACCTGCATTTCCGAATGTATCACCCCG
SEQ ID NO:5	(851)	TGAGATCAGATGCACCTATTGACACCTGCATTTCCGAATGTATCACCCCG
		901 950
SEQ ID NO:1	(901)	AACGGGAGCATCCCCAATGAAAAGCCCTTCCAAAATGTAAACAAGATCAC
SEQ ID NO:3	(901)	AACGGGAGCATCCCCAATGAAAAGCCCTTCCAAAATGTAAACAAGATCAC
SEQ ID NO:5	(901)	AACGGGAGCATCCCCAATGAAAAGCCCTTCCAAAATGTAAACAAGATCAC
		951 1000
SEQ ID NO:1	(951)	ATACGGAGCATGTCCCAAATATGTTAAGCAAAACACCTTGAAACTGGCAA
SEQ ID NO:3	(951)	ATACGGAGCATGTCCCAAATATGTTAAGCAAAACACCTTGAAACTGGCAA
SEQ ID NO:5	(951)	ATACGGAGCATGTCCCAAATATGTTAAGCAAAACACCTTGAAACTGGCAA
		1001 1050
SEQ ID NO:1	(1001)	CAGGAATGCGGAATGTCCCTGAGAGGCAAACCAGAGGCCTGTTCGGCGCA
SEQ ID NO:3	(1001)	CAGGAATGCGGAATGTCCCTGAGAGGCAAACCAGAGGCCTGTTCGGCGCA
SEQ ID NO:5	(1001)	CAGGAATGCGGAATGTCCCTGAGAGGCAAACCAGAGGCCTGTTCGGCGCA
		1051 1100
SEQ ID NO:1	(1051)	ATAGCAGGCTTCATAGAAAATGGATGGGAAGGGATGGTAGACGGTTGGTA
SEQ ID NO:3	(1051)	ATAGCAGGCTTCATAGAAAATGGATGGGAAGGGATGGTAGACGGTTGGTA
SEQ ID NO:5	(1051)	ATAGCAGGCTTCATAGAAAATGGATGGGAAGGGATGGTAGACGGTTGGTA
		1101 1150
SEQ ID NO:1	(1101)	TGGCTTCAGGCACCAAAATTCCGAAGGTACAGGACAAGCAGCAGACCTTA
SEQ ID NO:3	(1101)	TGGCTTCAGGCACCAAAATTCCGAAGGTACAGGACAAGCAGCAGACCTTA
SEQ ID NO:5	(1101)	TGGCTTCAGGCACCAAAATTCCGAAGGTACAGGACAAGCAGCAGACCTTA
		1151 1200
SEQ ID NO:1	(1151)	AAAGCACTCAGGCAGCCATTGACCAGATTAATGGGAAATTGAACAGAGTG
SEQ ID NO:3	(1151)	AAAGCACTCAGGCAGCCATTGACCAGATTAATGGGAAATTGAACAGAGTG
SEQ ID NO:5	(1151)	AAAGCACTCAGGCAGCCATTGACCAGATTAATGGGAAATTGAACAGAGTG
		1201 1250
SEQ ID NO:1	(1201)	ATTGAAAAAACGAATGAGAAGTTCCATCAAATTGAAAAGGAGTTTTCCGA
SEQ ID NO:3	(1201)	ATTGAAAAAACGAATGAGAAGTTCCATCAAATTGAAAAGGAGTTTTCCGA
SEQ ID NO:5	(1201)	ATTGAAAAAACGAATGAGAAGTTCCATCAAATTGAAAAGGAGTTTTCCGA
		1251 1300
SEQ ID NO:1	(1251)	AGTAGAAGGGAGGATTCAAGACCTTGAGAGATACGTTGAAGACACAAAAG
SEQ ID NO:3	(1251)	AGTAGAAGGGAGGATTCAAGACCTTGAGAGATACGTTGAAGACACAAAAG
SEQ ID NO:5	(1251)	AGTAGAAGGGAGGATTCAAGACCTTGAGAGATACGTTGAAGACACAAAAG

Figure 5D

```
                          1301                                              1350
SEQ ID NO:1   (1301)  TAGATCTTTGGTCTTACAATGCCGAGCTTCTTGTTGCCTTAGAAAACCAG
SEQ ID NO:3   (1301)  TAGATCTTTGGTCTTACAATGCCGAGCTTCTTGTTGCCTTAGAAAACCAG
SEQ ID NO:5   (1301)  TAGATCTTTGGTCTTACAATGCCGAGCTTCTTGTTGCCTTAGAAAACCAG
                      1351                                              1400
SEQ ID NO:1   (1351)  AACACAATTGATTTAACTGATTCAGAAATGAACAAATTGTTTGAAAAGAC
SEQ ID NO:3   (1351)  AACACAATTGATTTAACTGATTCAGAAATGAACAAATTGTTTGAAAAGAC
SEQ ID NO:5   (1351)  AACACAATTGATTTAACTGATTCAGAAATGAACAAATTGTTTGAAAAGAC
                      1401                                              1450
SEQ ID NO:1   (1401)  TAGGAGGCAATTGAGGGAAAAATGCTGAAGACATGGCAATGGCTGCTTCA
SEQ ID NO:3   (1401)  TAGGAGGCAATTGAGGGAAAAATGCTGAAGACATGGCAATGGCTGCTTCA
SEQ ID NO:5   (1401)  TAGGAGGCAATTGAGGGAAAAATGCTGAAGACATGGCAATGGCTGCTTCA
                      1451                                              1500
SEQ ID NO:1   (1451)  AGATATACCACAAGTGTGACAATGCTTGCATAGAATCGATTAGAAACGGA
SEQ ID NO:3   (1451)  AGATATACCACAAGTGTGACAATGCTTGCATAGAATCGATTAGAAACGGA
SEQ ID NO:5   (1451)  AGATATACCACAAGTGTGACAATGCTTGCATAGAATCGATTAGAAACGGA
                      1501                                              1550
SEQ ID NO:1   (1501)  ACTTATGACCATAACATATATAGAGATGAGGCAGTGAACAATCGGTTCCA
SEQ ID NO:3   (1501)  ACTTATGACCATAACATATATAGAGATGAGGCAGTGAACAATCGGTTCCA
SEQ ID NO:5   (1501)  ACTTATGACCATAACATATATAGAGATGAGGCAGTGAACAATCGGTTCCA
                      1551                                              1600
SEQ ID NO:1   (1551)  GATCAAAGGTGTTGAGCTAAAGTCTGGATACAAAGACTGGATCTTATGGA
SEQ ID NO:3   (1551)  GATCAAAGGTGTTGAGCTAAAGTCTGGATACAAAGACTGGATCTTATGGA
SEQ ID NO:5   (1551)  GATCAAAGGTGTTGAGCTAAAGTCTGGATACAAAGACTGGATCTTGTGGA
                      1601                                              1650
SEQ ID NO:1   (1601)  TTTCCTTTGCCATATCATGCTTTTTGCTTTGTGTTGTCTTGCTGGGTTTC
SEQ ID NO:3   (1601)  TTTCCTTTGCCATATCATGCTTTTTGCTTTGTGTTGTCTTGCTGGGTTTC
SEQ ID NO:5   (1601)  TTTCCTTTGCCATATCATGCTTTTTGCTTTGTGTTGTCTTGCTGGGTTTC
                      1651                                              1700
SEQ ID NO:1   (1651)  ATTATGTGGGCCTGCCAGAGAGGCAACATTAGGTGCAACATTTGCATTTG
SEQ ID NO:3   (1651)  ATTATGTGGGCCTGCCAGAGAGGCAACATTAGGTGCAACATTTGCATTTG
SEQ ID NO:5   (1651)  ATTATGTGGGCCTGCCAGAGAGGCAACATTAGGTGCAACATTTGCATTTG
                      1701
SEQ ID NO:1   (1701)  A
SEQ ID NO:3   (1701)  A
SEQ ID NO:5   (1701)  A
```

Sequence identity:
SEQ ID NO:1 v. SEQ ID NO:3: 99.9%
SEQ ID NO:1 v. SEQ ID NO:5: 99.9%
SEQ ID NO:3 v. SEQ ID NO:5: 99.8%

Figure 5E

Protein sequence alignment of HA protein of H3N8 and sequence identity

```
                        1                                                50
SEQ ID NO:31      (1)   MKTTIILILLTHWAYSQNPISGNNTATLCLGHHAVANGTLVKTMSDDQIE
SEQ ID NO:35      (1)   MKTTIILILLTHWAYSQNPISGNNTATLCLGHHAVANGTLVKTMSDDQIE
SEQ ID NO:39      (1)   MKTTIILILLTHWAYSQNPISGNNTATLCLGHHAVANGTLVKTMSDDQIE
                        51                                              100
SEQ ID NO:31     (51)   VTNATELVQSISMGKICNKSYRILDGRNCTLIDAMLGDPQCDAFQYESWD
SEQ ID NO:35     (51)   VTNVTELVQSISMGKICNKSYRILDGRNCTLIDAMLGDPQCDAFQYESWD
SEQ ID NO:39     (51)   VTNATELVQSISMGKICNKSYRILDGRNCTLIDAMLGDPHCDAFQYESWD
                        101                                             150
SEQ ID NO:31    (101)   LFIERSNAFSNCYPYDIPDYASLRSIVASSGTVEFTVEGFTWTGVTQNGR
SEQ ID NO:35    (101)   LFIERSNAFSNCYPYDIPDYASLRSIVASSGTVEFTAEGFTWTGVTQNGR
SEQ ID NO:39    (101)   LFIERSNAFSNCYPYDIPDYASLRSIVASSGTVKFTAEGFTWTGVTQNGR
                        151                                             200
SEQ ID NO:31    (151)   SGACKRGSADSFFSRLNWLTKSGSSYPTLNVTMPNNKNFDKLYIWGIHHP
SEQ ID NO:35    (151)   SGACKRGSADSFFSRLNWLTKSGSSYPTLNVTMPNNENFDKLYIWGIHHP
SEQ ID NO:39    (151)   SGACKRGSADSFFSRLNWLTKSGSSYPTLNVTMPNNKNFDKLYIWGIHHP
                        201                                             250
SEQ ID NO:31    (201)   SSNQEQTKLYIQESGRVTVSTKRSQQTIIPSIGSRPLVRGQSGRISIYWT
SEQ ID NO:35    (201)   SSNQEQTKLYIQESGRVTVSTKRSQQTIIPSIGSRPLIRGQSGRISIYWT
SEQ ID NO:39    (201)   SSNQEQTKLYIQESGRVTVSTKRSQQTIIPNIGSRPLWRGQSGRISIYWT
                        251                                             300
SEQ ID NO:31    (251)   IVKPGDILMINSNGNLVAPRGYFKLNRGKSSVIRSDVPIDICVSECITPN
SEQ ID NO:35    (251)   IVKPGDILMINSNGNLVAPRGYFKLNRGKSSVIRSDVPIDICVSECITPN
SEQ ID NO:39    (251)   IVKPGDILMINSNGNLVAPRGYFKLNTGKSSVMRSDVPIDICVSECITPN
                        301                                             350
SEQ ID NO:31    (301)   GSISNDKPFQNVNKVTYGKCPKYIRQNTLKLATGMRNVPEKQTRGIFGAI
SEQ ID NO:35    (301)   GSISNDKPFQNVNKVTYGKCPKYIRQNTLKLATGMRNVPEKQTRGIFGAI
SEQ ID NO:39    (301)   GSISNDKPFQNVNKVTYGKCPKYIRQNTLKLATGMRNVPEKQTRGIFGAI
                        351                                             400
SEQ ID NO:31    (351)   AGFIENGWEGMVDGWYGFRYQNSEGTGQAADLKSTQAAIDQINGKLNRVI
SEQ ID NO:35    (351)   AGFIENGWEGMVDGWYGFRYQNSEGTGQAADLKSTQAAIDQINGKLNRVI
SEQ ID NO:39    (351)   AGFIENGWEGMVDGWYGFRYQNSEGTGQAADLKSTQAAIDQINGKLNRVI
                        401                                             450
SEQ ID NO:31    (401)   ERTNEKFHQIEKEFSEVEERIQDLEKYVEDTKIDLWSYNAELLVALENQH
SEQ ID NO:35    (401)   ERTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQH
SEQ ID NO:39    (401)   ERTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQH
                        451                                             500
SEQ ID NO:31    (451)   TIDLTDAEMNKLFEKTRRQLRENAEDMGDGCFKIYHKCDNACIESIRNGT
SEQ ID NO:35    (451)   TIDLTDAEMNKLFEKTRRQLRENAEDMGNGCFKIYHKCDNACIESIRNGT
SEQ ID NO:39    (451)   TIDLTDAKMNKLFEKTRRQLRENAEDMGGGCFKIYHKCDNACIESIRNGT
                        501                                             550
SEQ ID NO:31    (501)   YDHYIYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGFI
SEQ ID NO:35    (501)   YDHYIYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGFI
SEQ ID NO:39    (501)   YDHYIYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLLCVVLLGFI
                        551          565
SEQ ID NO:31    (551)   MWACQKGNIRCNICI
SEQ ID NO:35    (551)   MWACQKGNIRCNICI
SEQ ID NO:39    (551)   MWACQKGNIRCNICI
```

Sequence identity

SEQ ID NO:31 v. SEQ ID NO:35: 98.9%

SEQ ID NO:31 v. SEQ ID NO:39: 97.9%

SEQ ID NO:35 v. SEQ ID NO:39: 97.7%

Figure 5F

DNA sequence alignment of polynucleotide encoding HA protein of H3N8 and sequence identity

```
                        1                                                50
SEQ ID NO:30    (1)     ATGAAAACAACCATTATTTTAATACTACTGACCCATTGGGCCTACAGTCA
SEQ ID NO:34    (1)     ATGAAAACAACCATTATTTTAATACTACTGACCCATTGGGCCTACAGTCA
SEQ ID NO:38    (1)     ATGAAGACAACCATTATTTTAATACTACTGACCCATTGGGCCCACAGTCA
                        51                                              100
SEQ ID NO:30    (51)    AAACCCAATCAGTGGCAATAACACAGCCACACTGTGTCTGGGACACCATG
SEQ ID NO:34    (51)    AAACCCAATCAGTGGCAATAACACAGCCACACTGTGTCTGGGACACCATG
SEQ ID NO:38    (51)    AAACCCAATCAGTGGCAATAACACAGCCACACTGTGTCTGGGACACCATG
                        101                                             150
SEQ ID NO:30    (101)   CAGTAGCAAATGGAACATTGGTAAAGACAATGAGTGATGATCAAATTGAG
SEQ ID NO:34    (101)   CAGTAGCAAATGGAACATTGGTAAAGACAATGAGTGATGATCAAATTGAG
SEQ ID NO:38    (101)   CAGTAGCAAATGGAACATTAGTAAAAACAATGAGTGATGATCAAATTGAG
                        151                                             200
SEQ ID NO:30    (151)   GTGACAAATGCTACAGAATTAGTTCAGAGCATTTCAATGGGGAAAATATG
SEQ ID NO:34    (151)   GTGACAAATGTTACAGAATTAGTTCAGAGCATTTCAATGGGGAAAATATG
SEQ ID NO:38    (151)   GTGACAAATGCTACAGAATTAGTTCAGAGCATTTCAATGGGGAAAATATG
                        201                                             250
SEQ ID NO:30    (201)   CAACAAATCATATAGAGTTCTAGATGGAAGAAATTGCACATTAATAGATG
SEQ ID NO:34    (201)   CAACAAATCATATAGAGTTCTAGATGGAAGAAATTGCACATTAATAGATG
SEQ ID NO:38    (201)   CAACAAATCATATAGAATTCTAGATGGAAGAAATTGCACATTAATAGATG
                        251                                             300
SEQ ID NO:30    (251)   CAATGCTAGGAGACCCCCATTGTGACGCCTTTCAGTATGAGAGTTGGGAC
SEQ ID NO:34    (251)   CAATGCTAGGAGACCCCCATTGTGACGCCTTTCAGTATGAGAGTTGGGAC
SEQ ID NO:38    (251)   CAATGCTAGGAGACCCCCACTGTGACGCCTTTCAGTATGAGAGTTGGGAC
                        301                                             350
SEQ ID NO:30    (301)   CTCTTTATAGAAAGAAGCAATGCTTTCAGCAATTGCTACCCATATGACAT
SEQ ID NO:34    (301)   CTCTTTATAGAAAGAAGCAACGCTTTCAGCAATTGCTACCCATATGACAT
SEQ ID NO:38    (301)   CTCTTTATAGAAAGAAGCAACGCTTTCAGCAATTGCTACCCATATGACAT
                        351                                             400
SEQ ID NO:30    (351)   CCCTGACTATGCATCGCTCCGATCCATTGTAGCATCCTCAGGAACAGTGG
SEQ ID NO:34    (351)   CCCTGACTATGCATCGCTCCGATCCATTGTAGCATCCTCAGGAACAGTGG
SEQ ID NO:38    (351)   CCCTGACTATGCATCGCTCCGATCCATTGTAGCATCCTCAGGAACAGTGA
                        401                                             450
SEQ ID NO:30    (401)   AATTCACAGTAGAGGGATTCACATGGACAGGTGTCACTCAAAACGGAAGA
SEQ ID NO:34    (401)   AATTCACAGCAGAGGGATTCACATGGACAGGTGTCACTCAAAACGGAAGA
SEQ ID NO:38    (401)   AATTCACAGCAGAGGGATTCACATGGACAGGTGTCACTCAAAACGGAAGA
                        451                                             500
SEQ ID NO:30    (451)   AGTGGAGCCTGCAAAAGGGGATCAGCCGATAGTTTCTTTAGCCGACTGAA
SEQ ID NO:34    (451)   AGTGGAGCCTGCAAAAGGGGATCAGCCGATAGTTTCTTTAGCCGACTGAA
SEQ ID NO:38    (451)   AGTGGAGCCTGCAAAAGGGGATCAGCCGATAGTTTCTTTAGCCGACTGAA
                        501                                             550
SEQ ID NO:30    (501)   TTGGCTAACAAAATCTGGAAGCTCTTACCCCACATTGAATGTGACAATGC
SEQ ID NO:34    (501)   TTGGCTAACAAAATCTGGAAGCTCTTACCCCACATTGAATGTAACAATGC
SEQ ID NO:38    (501)   TTGGCTAACAAAATCTGGAAGCTCTTACCCCACATTGAATGTGACAATGC
                        551                                             600
SEQ ID NO:30    (551)   CTAACAATAAAAATTTCGACAAGCTATACATCTGGGGGATTCATCACCCG
SEQ ID NO:34    (551)   CTAACAATGAAAATTTCGACAAGCTATACATCTGGGGGATTCATCACCCG
SEQ ID NO:38    (551)   CTAACAATAAAAATTTCGACAAGCTATACATCTGGGGGATTCATCACCCA
                        601                                             650
SEQ ID NO:30    (601)   AGCTCGAATCAAGAGCACACAAAATTGTACATCCAAGAATCAGGACGAGT
SEQ ID NO:34    (601)   AGCTCGAATCAAGAGCAGACAAAATTGTACATTCAAGAATCAGGACGAGT
SEQ ID NO:38    (601)   AGCTCAAATCAAGAGCAGACAAAATTGTACATCCAAGAATCAGGACGAGT
```

Figure 5G

```
                         651                                              700
SEQ ID NO:30    (651)  AACAGTCTCAACAAAAAGAAGTCAACAAACAATAATCCCTGACATCGGAT
SEQ ID NO:34    (651)  AACAGTCTCAACAAAAAGAAGTCAACAAACAATAATTCCTGACATCGGAT
SEQ ID NO:38    (651)  AACAGTCTCAACAAAAAGAAGTCAACAAACAATAATCCCTAACATCGGAT
                         701                                              750
SEQ ID NO:30    (701)  CTAGACCGTTGATCAGAGGTCAATCAGGCAGGATAAGCATATACTGGACC
SEQ ID NO:34    (701)  CTAGACCGTTGATCAGAGGTCAATCAGGCAGGATAAGCATATACTGGACC
SEQ ID NO:38    (701)  CTAGACCGTTGGTCAGAGGTCAATCAGGCAGGATAAGCATATACTGGACC
                         751                                              800
SEQ ID NO:30    (751)  ATTGTAAAACCTGGAGATATCCTAATGATAAACAGTAATGGCAACTTAGT
SEQ ID NO:34    (751)  ATTGTAAAACCTGGAGATATCCTAATGATAAACAGTAATGGCAACTTAGT
SEQ ID NO:38    (751)  ATTGTAAAACCTGGAGATATCCTAATGATAAACAGTAATGGCAACTTAGT
                         801                                              850
SEQ ID NO:30    (801)  TGCACCGCGGGGATATTTCAAATTGAACCCAGGAAAAAGCTCTGTAATCA
SEQ ID NO:34    (801)  TGCACCGCGGGGATATTTCAAATTGAACCCAGGAAAAAGCTCTGTAATTA
SEQ ID NO:38    (801)  TGCACCGCGGGGATATTTTAAACTGAACACAGGGAAAAGCTCTGTAATCA
                         851                                              900
SEQ ID NO:30    (851)  GATCCGATGTACCCATAGACATTTGTGTGTCTGAATGTATTACACCAAAT
SEQ ID NO:34    (851)  GATCCGATGTACCCATAGACATTTGTGTGTCTGAATGTATTACACCAAAT
SEQ ID NO:38    (851)  GATCCGATGTACCCATAGACATTTGTGTGTCTGAATGTATTACACCAAAT
                         901                                              950
SEQ ID NO:30    (901)  GGAAGCATCTCCAACGACAAGCCATTCCAAAATGTGAACAAAGTTACATA
SEQ ID NO:34    (901)  GGAAGCATCTCCAACGACAAGCCATTCCAAAATGTGAACAAAGTTACATA
SEQ ID NO:38    (901)  GGAAGCATCTCCAACGACAAGCCATTCCAAAATGTGAACAAAGTTACATA
                         951                                             1000
SEQ ID NO:30    (951)  TGGAAAATGCCCCAAGTATATCAGGCAAAACACTTTAAAGTTGGCCACTG
SEQ ID NO:34    (951)  TGGAAAATGCCCCAAGTATATCAGGCAAAACACTTTAAAGTTGGCCACTG
SEQ ID NO:38    (951)  TGGAAAATGCCCCAAGTATATCAGGCAAAACACTTTAAAGCTGGCCACTG
                         1001                                            1050
SEQ ID NO:30   (1001)  GGATGAGGAATGTGCCAGAAAAGCAAACCAGAGGAATCTTTGGGGCGATA
SEQ ID NO:34   (1001)  GGATGAGGAATGTGCCAGAAAAGCAAACCAGAGGAATCTTTGGGGCGATA
SEQ ID NO:38   (1001)  GGATGAGGAATGTACCAGAAAAGCAAACCAGAGGAATCTTTGGAGCAATA
                         1051                                            1100
SEQ ID NO:30   (1051)  GCGGGATTCATCGAAAACGGCTGGGAAGGAATGGTTGATGGGTGGTATGG
SEQ ID NO:34   (1051)  GCGGGATTCATCGAAAACGGCTGGGAAGGAATGGTTGATGGGTGGTATGG
SEQ ID NO:38   (1051)  GCGGGATTCATCGAAAACGGCTGGGAAGGAATGGTTGATGGGTGGTATGG
                         1101                                            1150
SEQ ID NO:30   (1101)  GTTCCGATATCAAAACTCTGAAGGAACAGGGCAAGCTGCAGATCTAAAGA
SEQ ID NO:34   (1101)  GTTCCGATATCAAAACTCTGAAGGAACAGGGCAAGCTGCAGATCTAAAGA
SEQ ID NO:38   (1101)  GTTCCGATATCAAAACTCTGAAGGAACAGGGCAAGCTGCAGATCTAAAGA
                         1151                                            1200
SEQ ID NO:30   (1151)  GCACTCAAGCAGCCATCGACCAGATTAATGGAAAGTTAAACAGGCTGATT
SEQ ID NO:34   (1151)  GCACTCAAGCAGCCATCGACCAGATTAATGGAAAGTTAAACAGGCTGATT
SEQ ID NO:38   (1151)  GCACTCAAGCAGCCATCGACCAGATTAATGGAAAGTTAAACAGACTGATT
                         1201                                            1250
SEQ ID NO:30   (1201)  GAAAGAACCAATGAGAAATTCCATCAAATAGAGAAGGAATTCTCAGAAGT
SEQ ID NO:34   (1201)  GAAAGAACCAATGAGAAATTCCATCAAATAGAGAAGGAATTCTCAGAAGT
SEQ ID NO:38   (1201)  GAAAGAACCAATGAGAAATTCCATCAAATAGAGAAGGAATTCTCAGAAGT
                         1251                                            1300
SEQ ID NO:30   (1251)  AGAAGAAAGAATTCAGGACTTGGAGAAATATGTAGAAGACACCAAAATAG
SEQ ID NO:34   (1251)  AGAAGGAAGAATTCAGGACTTGGAGAAATATGTAGAAGACACCAAAATAG
SEQ ID NO:38   (1251)  AGAAGGAAGAATTCAGGACTTGGAGAAATATGTAGAAGACACCAAAATAG
```

Figure 5H

```
                         1301                                              1350
SEQ ID NO:30   (1301)    ACCTATGGTCCTACAATGCAGAACTGCTGGTGGCTCTAGAAAATCAACAT
SEQ ID NO:34   (1301)    ACCTATGGTCCTACAATGCAGAACTACTGGTGGCTCTAGAAAATCAACAT
SEQ ID NO:38   (1301)    ACCTATGGTCCTACAATGCAGAATTGCTGGTGGCTCTAGAAAATCAACAT
                         1351                                              1400
SEQ ID NO:30   (1351)    ACAATTGACTTAACAGATGCAGAAATGAATAAATTATTTGAGAAGACTAG
SEQ ID NO:34   (1351)    ACAATTGACTTAACAGATGCAGAAATGAATAAATTATTTGAGAAGACTAG
SEQ ID NO:38   (1351)    ACAATTGACTTAACAGATGCAAAAATGAATAAATTATTTGAGAAGACTAG
                         1401                                              1450
SEQ ID NO:30   (1401)    ACGCCAGTTAAGAGAAAATGCAGAAGACATGGAGATGGATGTTTCAAGA
SEQ ID NO:34   (1401)    ACGCCAGTTAAGAGAAAATGCAGAAGACATGGAAATGGATGTTTCAAGA
SEQ ID NO:38   (1401)    ACGCCAGTTGAGAGAAAACGCAGAAGACATGGAGGTGGATGTTTCAAGA
                         1451                                              1500
SEQ ID NO:30   (1451)    TTTACCACAAGTGTGATAATGCATGCATTGAATCAATAAGAACTGGAACA
SEQ ID NO:34   (1451)    TTTACCACAAGTGTGATAATGCATGCATTGAGTCAATAAGAACTGGAACA
SEQ ID NO:38   (1451)    TTTATCACAAATGTGATAATGCATGCATTGAATCAATAAGAACTGGAACA
                         1501                                              1550
SEQ ID NO:30   (1501)    TATGACCATTACATATACAGAGATGAAGCAATAAACAACCGATTTCAGAT
SEQ ID NO:34   (1501)    TATGACCATTACATATACAGAGATGAAGCAATAAACAACCGATTTCAGAT
SEQ ID NO:38   (1501)    TATGACCATTACATATACAGAGATGAAGCATTAAACAACCGATTTCAGAT
                         1551                                              1600
SEQ ID NO:30   (1551)    CAAAGGTGTAGAATTGAAATCAGGCTACAAAGATTGGATACTGTGGATTT
SEQ ID NO:34   (1551)    CAAAGGTGTAGAATTGAAATCAGGCTACAAAGATTGGATACTGTGGATTT
SEQ ID NO:38   (1551)    CAAAGGTGTAGAGTTGAAATCAGGCTACAAAGATTGGATACTGTGGATTT

                         1601                                              1650
SEQ ID NO:30   (1601)    CATTCGCCATATCATGCTTCTTAATTTGCGTTGTTCTATTGGGTTTCATT
SEQ ID NO:34   (1601)    CATTCGCCATATCATGCTTCTTAATTTGCGTTGTTCTATTGGGTTTCATT
SEQ ID NO:38   (1601)    CATTCGCCATATCATGCTTCTTAATTTGCGTTGTTCTATTGGGTTTCATT
                         1651                                         1695
SEQ ID NO:30   (1651)    ATGTGGCTTGCCAAAAAGGCAACATCAGATGCAACATTTGCATT
SEQ ID NO:34   (1651)    ATGTGGCTTGCCAAAAAGGCAACATCAGATGCAACATTTGCATT
SEQ ID NO:38   (1651)    ATGTGGCTTGCCAAAAAGGCAACATCAGATGCAACATTTGCATT
```

Sequence identity:
SEQ ID NO:30 v. SEQ ID NO:34: 99.3%
SEQ ID NO:30 v. SEQ ID NO:38: 98.1%
SEQ ID NO:34 v. SEQ ID NO:38: 97.8%

Sequence identity between HA proteins of H3N2 and H3N8

SEQ ID NO:	2	4	6	31	35	39
2		99.6%	99.6%	84.4%	84.8%	85.4%
4			99.6%	84.8%	84.8%	85.3%
6				85.0%	85.0%	85.5%
31					98.9%	97.9%
35						97.7%
39						

Figure 5I

Protein sequence alignment of NA protein of H3N2 and sequence identity

```
                     1                                                 50
SEQ ID NO:8     (1)  MNPNQKIIAIGSVSLTIATVCFLLQIAILATTVTLYFKQNECNIPSNSQV
SEQ ID NO:10    (1)  MNPNQKIIAIGSVSLTIATVCFLLQIAILATTVTLYFKQNECNIPSNSQV
SEQ ID NO:12    (1)  MNPNQKIIAIGSVSLTIATVCFLLQIAILATTVTLYFKQNECNIPSNSQV
                     51                                               100
SEQ ID NO:8    (51)  VPCKPIIIERNITEVVYLNNTTIEKEICSVVLEYRNWSKPQCQITGFAPF
SEQ ID NO:10   (51)  VPCKPIIIERNITEVVYLNNTTIEKEICSVVLEYRNWSKPQCQITGFAPF
SEQ ID NO:12   (51)  VPCKPIIIERNITEVVYLNNTTIEKEICSVVLEYRNWSKPQCQITGFAPF
                     101                                              150
SEQ ID NO:8   (101)  SKDNSIRLSAGGDIWVTREPYVSCDHSKCYQFALGQGTTLNNKHSNSTIH
SEQ ID NO:10  (101)  SKDNSIRLSAGGDIWVTREPYVSCDHSKCYQFALGQGTTLNNKHSNSTIH
SEQ ID NO:12  (101)  SKDNSIRLSAGGDIWVTREPYVSCDHSKCYQFALGQGTTLNNKHSNSTIH
                     151                                              200
SEQ ID NO:8   (151)  DRTSHRTLLMNELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCVTGDDRN
SEQ ID NO:10  (151)  DRTSHRTLLMNELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCVTGDDRN
SEQ ID NO:12  (151)  DRTSHRTLLMNELGVPFHLGTKQVCIAWSSSSCHDGKAWLHVCVTGDDRN
                     201                                              250
SEQ ID NO:8   (201)  ATASFVYNGMLVDSIGSWSRNILRTQESECVCINGTCTVVMTDGSASGRA
SEQ ID NO:10  (201)  ATASFVYNGMLVDSIGSWSRNILRTQESECVCINGTCTVVMTDGSASGRA
SEQ ID NO:12  (201)  ATASFVYNGMLVDSIGSWSRNILRTQESECVCINGTCTVVMTDGSASGRA
                     251                                              300
SEQ ID NO:8   (251)  DTRILFIREGKIIHISPLSGSAQHIEECSCYPRYPNVRCVCRDNWKGSNR
SEQ ID NO:10  (251)  DTRILFIREGKIIHISPLSGSAQHIEECSCYPRYPNVRCVCRDNWKGSNR
SEQ ID NO:12  (251)  DTRILFIREGKIIHISPLSGSAQHIEECSCYPRYPNVRCVCRDNWKGSNR
                     301                                              350
SEQ ID NO:8   (301)  PVIDINMADYNINSSYVCSGLVGDTPRNDDSSSSSNCKDPNNERGNPGVK
SEQ ID NO:10  (301)  PVIDINMADYNINSSYVCSGLVGDTPRNDDSSSSSNCKDPNNERGNPGVK
SEQ ID NO:12  (301)  PVIDINMADYNINSSYVCSGLVGDTPRNDDSSSSSNCKDPNNERGNPGVK
                     351                                              400
SEQ ID NO:8   (351)  GWAFDNDNDVWMGRTISKDLRSGYETFKVIGGWTTANSKSQVNRQVIVDN
SEQ ID NO:10  (351)  GWAFDNDNDVWMGRTISKDLRSGYETFKVIGGWTTANSKSQVNRQVIVDN
SEQ ID NO:12  (351)  GWAFDNDNDVWMGRTISKDLRSGYETFKVIGGWTTANSKSQVNRQVIVDN
                     401                                              450
SEQ ID NO:8   (401)  NNWSGYSGIFSVEGKSCVNRCFYVELIRGGPQETRVWWTSNSIVVFCGTS
SEQ ID NO:10  (401)  NNWSGYSGIFSVEGKSCVNRCFYVELIRGGPQETRVWWTSNSIVVFCGTS
SEQ ID NO:12  (401)  NNWSGYSGIFSVEGKSCVNRCFYVELIRGGPQETRVWWTSNSIVVFCGTS
                     451               469
SEQ ID NO:8   (451)  GTYGTGSWPDGANINFMPI
SEQ ID NO:10  (451)  GTYGTGSWPDGANINFMPI
SEQ ID NO:12  (451)  GTYGTGSWPDGANINFMPI
```

```
Sequence identity
SEQ ID NO:8 v. SEQ ID NO:10:  100.0%
SEQ ID NO:8 v. SEQ ID NO:12: 100.0%
SEQ ID NO:10 v. SEQ ID NO:12: 100.0%
```

Figure 5J

DNA sequence alignment of polynucleotide encoding NA protein of H3N2 and sequence identity

```
                      1                                                50
SEQ ID NO:7     (1)   ATGAACCCAAATCAAAAGATAATAGCAATAGGGTCTGTCTCTCTAACCAT
SEQ ID NO:9     (1)   ATGAACCCAAATCAAAAGATAATAGCAATAGGGTCTGTCTCTCTAACCAT
SEQ ID NO:11    (1)   ATGAACCCAAATCAAAAGATAATAGCAATAGGGTCTGTCTCTCTAACCAT
                      51                                              100
SEQ ID NO:7    (51)   TGCAACAGTATGTTTCCTCTTACAGATTGCCATCCTAGCAACAACTGTGA
SEQ ID NO:9    (51)   TGCAACAGTATGTTTCCTCTTACAGATTGCCATCCTAGCAACAACTGTGA
SEQ ID NO:11   (51)   TGCAACAGTATGTTTCCTCTTACAGATTGCCATCCTAGCAACAACTGTGA
                      101                                             150
SEQ ID NO:7   (101)   CACTGTACTTCAAGCAAAATGAATGCAACATCCCCTCGAACAGTCAAGTA
SEQ ID NO:9   (101)   CACTGTACTTCAAGCAAAATGAATGCAACATCCCCTCGAACAGTCAAGTA
SEQ ID NO:11  (101)   CACTGTACTTCAAGCAAAATGAATGCAACATCCCCTCGAACAGTCAAGTA
                      151                                             200
SEQ ID NO:7   (151)   GTGCCATGTAAACCAATCATAATAGAAAGGAACATAACAGAGGTAGTATA
SEQ ID NO:9   (151)   GTGCCATGTAAACCAATCATAATAGAAAGGAACATAACAGAGGTAGTATA
SEQ ID NO:11  (151)   GTGCCATGTAAACCAATCATAATAGAAAGGAACATAACAGAGGTAGTATA
                      201                                             250
SEQ ID NO:7   (201)   TTTGAATAATACTACCATAGAAAAAGAAATTTGCTCCGTAGTGCTAGAAT
SEQ ID NO:9   (201)   TTTGAATAATACTACCATAGAAAAAGAAATTTGCTCCGTAGTGCTAGAAT
SEQ ID NO:11  (201)   TTTGAATAATACTACCATAGAAAAAGAAATTTGCTCCGTAGTGCTAGAAT
                      251                                             300
SEQ ID NO:7   (251)   ACAGGAACTGGTCGAAACCGCAGTGTCAAATTACAGGATTTGCTCCTTTC
SEQ ID NO:9   (251)   ACAGGAACTGGTCGAAACCGCAGTGTCAAATTACAGGATTTGCTCCTTTC
SEQ ID NO:11  (251)   ACAGGAACTGGTCGAAACCGCAGTGTCAAATTACAGGATTTGCTCCTTTC
                      301                                             350
SEQ ID NO:7   (301)   TCCAAGGACAACTCAATCCGACTCTCCGCTGGTGGGGACATTTGGGTAAC
SEQ ID NO:9   (301)   TCCAAGGACAACTCAATCCGACTCTCCGCTGGTGGGGACATTTGGGTAAC
SEQ ID NO:11  (301)   TCCAAGGACAACTCAATCCGACTCTCCGCTGGTGGGGACATTTGGGTAAC
                      351                                             400
SEQ ID NO:7   (351)   AAGGGAACCTTATGTGTCATGCGACCACAGCAAATGTTATCAGTTTGCAC
SEQ ID NO:9   (351)   AAGGGAACCTTATGTGTCATGCGACCACAGCAAATGTTATCAGTTTGCAC
SEQ ID NO:11  (351)   AAGGGAACCTTATGTGTCATGCGACCACAGCAAATGTTATCAGTTTGCAC
                      401                                             450
SEQ ID NO:7   (401)   TTGGGCAGGGGACCACGCTGAACAATAAACACTCAAACAGCACAATACAT
SEQ ID NO:9   (401)   TTGGGCAGGGGACCACGCTGAACAATAAACACTCAAACAGCACAATACAT
SEQ ID NO:11  (401)   TTGGGCAGGGGACCACGCTGAACAATAAACACTCAAACAGCACAATACAT
                      451                                             500
SEQ ID NO:7   (451)   GATAGGACCTCTCATCGAACTCTTTTAATGAATGAGTTGGGTGTTCCGTT
SEQ ID NO:9   (451)   GATAGGACCTCTCATCGAACTCTTTTAATGAATGAGTTGGGTGTTCCGTT
SEQ ID NO:11  (451)   GATAGGACCTCTCATCGAACTCTTTTAATGAATGAGTTGGGTGTTCCGTT
                      501                                             550
SEQ ID NO:7   (501)   TCATTTGGGAACCAAACAAGTGTGCATAGCATGGTCCAGTTCAAGTTGTC
SEQ ID NO:9   (501)   TCATTTGGGAACCAAACAAGTGTGCATAGCATGGTCCAGTTCAAGTTGTC
SEQ ID NO:11  (501)   TCATTTGGGAACCAAACAAGTGTGCATAGCATGGTCCAGTTCAAGTTGTC
                      551                                             600
SEQ ID NO:7   (551)   ACGATGGGAAAGCATGGTTACATGTTTGTGTCACTGGAGATGATAGAAAT
SEQ ID NO:9   (551)   ACGATGGGAAAGCATGGTTACATGTTTGTGTCACTGGAGATGATAGAAAT
SEQ ID NO:11  (551)   ACGATGGGAAAGCATGGTTACATGTTTGTGTCACTGGAGATGATAGAAAT
                      601                                             650
SEQ ID NO:7   (601)   GCGACTGCTAGTTTCGTTTATAATGGAATGCTTGTTGACAGTATTGGTTC
SEQ ID NO:9   (601)   GCGACTGCTAGTTTCGTTTATAATGGAATGCTTGTTGACAGTATTGGTTC
SEQ ID NO:11  (601)   GCGACTGCTAGTTTCGTTTATAATGGAATGCTTGTTGACAGTATTGGTTC
```

Figure 5K

```
                      651                                              700
SEQ ID NO:7    (651)  ATGGTCTCGAAATATCCTCAGAACTCAAGAGTCAGAATGTGTTTGCATCA
SEQ ID NO:9    (651)  ATGGTCTCGAAATATCCTCAGAACTCAAGAGTCAGAATGTGTTTGCATCA
SEQ ID NO:11   (651)  ATGGTCTCGAAATATCCTCAGAACTCAAGAGTCAGAATGTGTTTGCATCA
                      701                                              750
SEQ ID NO:7    (701)  ATGGGACTTGTACAGTAGTAATGACTGATGGAAGTGCATCAGGAAGGGCT
SEQ ID NO:9    (701)  ATGGGACTTGTACAGTAGTAATGACTGATGGAAGTGCATCAGGAAGGGCT
SEQ ID NO:11   (701)  ATGGGACTTGTACAGTAGTAATGACTGATGGAAGTGCATCAGGAAGGGCT
                      751                                              800
SEQ ID NO:7    (751)  GATACTAGAATACTATTCATCAGAGAGGGGAAAATTATCCATATTAGCCC
SEQ ID NO:9    (751)  GATACTAGAATACTATTCATCAGAGAGGGGAAAATTATCCATATTAGCCC
SEQ ID NO:11   (751)  GATACTAGAATACTATTCATCAGAGAGGGGAAAATTATCCATATTAGCCC
                      801                                              850
SEQ ID NO:7    (801)  ATTGTCAGGGAGTGCTCAACACATAGAGGAATGTTCCTGTTATCCCCGAT
SEQ ID NO:9    (801)  ATTGTCAGGGAGTGCTCAACACATAGAGGAATGTTCCTGTTATCCCCGAT
SEQ ID NO:11   (801)  ATTGTCAGGGAGTGCTCAACACATAGAGGAATGTTCCTGTTATCCCCGAT
                      851                                              900
SEQ ID NO:7    (851)  ATCCAAATGTTAGATGTGTTTGCAGAGACAATTGGAAGGGCTCCAATAGG
SEQ ID NO:9    (851)  ATCCAAATGTTAGATGTGTTTGCAGAGACAATTGGAAGGGCTCCAATAGG
SEQ ID NO:11   (851)  ATCCAAATGTTAGATGTGTTTGCAGAGACAATTGGAAGGGCTCCAATAGG
                      901                                              950
SEQ ID NO:7    (901)  CCCGTTATAGATATAAATATGGCAGATTATAACATCAATTCCAGTTATGT
SEQ ID NO:9    (901)  CCCGTTATAGATATAAATATGGCAGATTATAACATCAATTCCAGTTATGT
SEQ ID NO:11   (901)  CCCGTTATAGATATAAATATGGCAGATTATAACATCAATTCCAGTTATGT
                      951                                             1000
SEQ ID NO:7    (951)  CTGTTCAGGACTTGTTGGCGATACACCAAGGAATGATGATAGCTCTAGCA
SEQ ID NO:9    (951)  CTGTTCAGGACTTGTTGGCGATACACCAAGGAATGATGATAGCTCTAGCA
SEQ ID NO:11   (951)  CTGTTCAGGACTTGTTGGCGATACACCAAGGAATGATGATAGCTCTAGCA
                      1001                                            1050
SEQ ID NO:7   (1001)  GCAGTAACTGCAAGGATCCTAATAATGAGAGAGGGAATCCAGGAGTGAAG
SEQ ID NO:9   (1001)  GCAGTAACTGCAAGGATCCTAATAATGAGAGAGGGAATCCAGGAGTGAAG
SEQ ID NO:11  (1001)  GCAGTAACTGCAAGGATCCTAATAATGAGAGAGGGAATCCAGGAGTGAAG
                      1051                                            1100
SEQ ID NO:7   (1051)  GGGTGGGCCTTTGATAATGATAATGACGTTTGGATGGGGAGGACAATCAG
SEQ ID NO:9   (1051)  GGGTGGGCCTTTGATAATGATAATGACGTTTGGATGGGGAGGACAATCAG
SEQ ID NO:11  (1051)  GGGTGGGCCTTTGATAATGATAATGACGTTTGGATGGGGAGGACAATCAG
                      1101                                            1150
SEQ ID NO:7   (1101)  CAAAGATTTACGTTCAGGTTATGAGACTTTCAAGGTCATTGGTGGCTGGA
SEQ ID NO:9   (1101)  CAAAGATTTACGTTCAGGTTATGAGACTTTCAAGGTCATTGGTGGCTGGA
SEQ ID NO:11  (1101)  CAAAGATTTACGTTCAGGTTATGAGACTTTCAAGGTCATTGGTGGCTGGA
                      1151                                            1200
SEQ ID NO:7   (1151)  CCACTGCTAATTCCAAGTCACAGGTCAATAGACAAGTCATAGTTGACAAT
SEQ ID NO:9   (1151)  CCACTGCTAATTCCAAGTCACAGGTCAATAGACAAGTCATAGTTGACAAT
SEQ ID NO:11  (1151)  CCACTGCTAATTCCAAGTCACAGGTCAATAGACAAGTCATAGTTGACAAT
                      1201                                            1250
SEQ ID NO:7   (1201)  AATAACTGGTCTGGTTATTCTGGTATTTTCTCCGTTGAAGGCAAAAGCTG
SEQ ID NO:9   (1201)  AATAACTGGTCTGGTTATTCTGGTATTTTCTCCGTTGAAGGCAAAAGCTG
SEQ ID NO:11  (1201)  AATAACTGGTCTGGTTATTCTGGTATTTTCTCCGTTGAAGGCAAAAGCTG
                      1251                                            1300
SEQ ID NO:7   (1251)  TGTTAATAGGTGTTTTTATGTGGAGTTGATAAGAGGAGGGCCACAAGACA
SEQ ID NO:9   (1251)  TGTTAATAGGTGTTTTTATGTGGAGTTGATAAGAGGAGGGCCACAAGACA
SEQ ID NO:11  (1251)  TGTTAATAGGTGTTTTTATGTGGAGTTGATAAGGGGAGGGCCACAAGACA
```

Figure 5L

```
                              1301                                             1350
SEQ ID NO:7     (1301)  CTAGAGTATGGTGGACTTCAAATACCATTGTCGTATTTTGTGGTACTTCT
SEQ ID NO:9     (1301)  CTAGAGTATGGTGGACTTCAAATACCATTGTCGTATTTTGTGGTACTTCT
SEQ ID NO:11    (1301)  CTAGAGTATGGTGGACTTCAAATACCATTGTCGTATTTTGTGGTACTTCT
                              1351                                             1400
SEQ ID NO:7     (1351)  GGTACCTATGGAACAGGCTCATGGCCTGATGGGGCGAATATTAACTTCAT
SEQ ID NO:9     (1351)  GGTACCTATGGAACAGGCTCATGGCCTGATGGGGCGAATATTAACTTCAT
SEQ ID NO:11    (1351)  GGTACCTATGGAACAGGCTCATGGCCTGATGGAGCGAATATTAACTTCAT
                              1401
SEQ ID NO:7     (1401)  GCCTATATAA
SEQ ID NO:9     (1401)  GCCTATATAA
SEQ ID NO:11    (1401)  GCCTATATAA
```

Sequence identity
SEQ ID NO:7 v. SEQ ID NO:9: 100.0%
SEQ ID NO:7 v. SEQ ID NO:11: 99.9%
SEQ ID NO:9 v. SEQ ID NO:11: 99.9%

DNA sequence alignment of polynucleotide encoding NA protein of H3N8 and sequence identity

```
                              1                                                  50
SEQ ID NO:32       (1)  ATGAACCCAAATCAAAAGATAATAGCAATTGGATCTGCATCATTGGGGAT
SEQ ID NO:36       (1)  ATGAATCCAAATCAAAAGATAATAGCAATTGGATCTGCATCATTGGGGAT
SEQ ID NO:40       (1)  ATGAATCCAAATCAAAAGATAATAGCAATTGGATTTGCATCATTGGGGAT
SEQ ID NO:42       (1)  ATGAACCCAAATCAAAAGATAATAGCAATTGGATCTGCATCATTGGGGAT
                              51                                                100
SEQ ID NO:32      (51)  ATTAATCATTAATATCATTCTCCATGTAGTCAGCATTATAGTAACAGTAC
SEQ ID NO:36      (51)  ATTAATCATTAATATCATTCTCCATGTAGTCAGCATTATAGTAACAGTAC
SEQ ID NO:40      (51)  ATTAATCATTAATGTCATTCTCCATGTAGTCAGCATTATAGTAACAGTAC
SEQ ID NO:42      (51)  ATTAATCATTAATATCATTCTCCATGTAGTCAGCATTATAGTAACAGTAC
                              101                                               150
SEQ ID NO:32     (101)  TGGTCCTCAATAACAATAGAACAGATCTGAACTGCAAAGGCACGATCATA
SEQ ID NO:36     (101)  TGGTCCTCAATAACAATAGAACAGATCTGAACTGCAAAGGCACGATCATA
SEQ ID NO:40     (101)  TGGTCCTCAATAACAATAGAACAGATCTGAACTACAAAGGCACGATCATA
SEQ ID NO:42     (101)  TGGTCCTCAATAACAATAGAACAGATCTGAACTGCAAAGGCACGATCATA
                              151                                               200
SEQ ID NO:32     (151)  AGAGAGTACAATGAAACAGTAAGAGTAGAAAAACTTACTCAATGGTACAA
SEQ ID NO:36     (151)  AGAGAGTACAATGAAACAGTAAGAGTAGAAAAACTTACTCAATGGTATAA
SEQ ID NO:40     (151)  AGAGAATACAATGAAACAGTAAGAGTAGAAAAACTTACTCAATGGTATAA
SEQ ID NO:42     (151)  AGAGAGTACAATGAAACAGTAAGAGTAGAAAAACTTACTCAATGGTATAA
                              201                                               250
SEQ ID NO:32     (201)  CATCAGTACAACCAAGTACATAGAGAGACCTTCAAATGAATATTACATGA
SEQ ID NO:36     (201)  TATCAGTACAATTAAGTACATAGAGAGACCTTCAAATGAATATTACATGA
SEQ ID NO:40     (201)  TACCAGTACAATTAAGTACATAGAGAGACCTTCAAATGAATACTACATGA
SEQ ID NO:42     (201)  TATCAGTACAATTAAGTACATAGAGAGACCTTCAAATGAATATTACATGA
                              251                                               300
SEQ ID NO:32     (251)  ACAACACTGAACCACTTTGTGAGGCCCAAGGCTTTGCACCATTTTCCAAA
SEQ ID NO:36     (251)  ACAACACTGAACCACTTTGTGAGGCCCAAGGCTTTGCACCATTTTCCAAA
SEQ ID NO:40     (251)  ATAACACTGAACCACTTTGTGAGGCCCAAGGCTTTGCACCATTTTCCAAA
SEQ ID NO:42     (251)  ACAACACTGAACCACTTTGTGAGGCCCAAGGCTTTGCACCATTTTCCAAA
```

Figure 5M

```
                          301                                              350
SEQ ID NO:32      (301)   GATAATGGAATACGAATTGGGTCGAGAGGCCATGTTTTTGTGATAAGAGA
SEQ ID NO:36      (301)   GATAATGGAATACGAATTGGGTCGAGAGGCCATGTTTTTGTGATAAGAGA
SEQ ID NO:40      (301)   GATAATGGAATACGAATTGGGTCGAGAGGCCATGTTTTTGTGATAAGAGA
SEQ ID NO:42      (301)   GATAATGGAATACGAATTGGGTCGAGAGGCCATGTTTTTGTGATAAGAGA
                          351                                              400
SEQ ID NO:32      (351)   ACCTTTTGTATCATGTTCACCCTCAGAATGTAGAACCTTTTTCCTCACAC
SEQ ID NO:36      (351)   ACCTTTTGTATCATGTTCACCCTCAGAATGTAGAACCTTTTTCCTCACAC
SEQ ID NO:40      (351)   ACCTTTTGTATCATGTTCGCCCTCAGAATGTAGAACCTTTTTCCTCACAC
SEQ ID NO:42      (351)   ACCTTTTGTATCATGTTCACCCTCAGAATGTAGAACCTTTTTCCTCACAC
                          401                                              450
SEQ ID NO:32      (401)   AGGGCTCATTACTCAATGACAAACATTCTAACGGCACAATAAAGGATCGA
SEQ ID NO:36      (401)   AGGGCTCATTACTCAATGACAAACATTCTAACGGCACAATAAAGGATCGA
SEQ ID NO:40      (401)   AGGGCTCATTACTCAATGACAAACATTCTAACGGCACAATAAAGGATCGA
SEQ ID NO:42      (401)   AGGGCTCATTACTCAATGACAAACATTCTAACGGCACAATAAAGGATCGA
                          451                                              500
SEQ ID NO:32      (451)   AGTCCGTATAGGACTCTGATGAGTGTCAAAATAGGGCAATCACCTAATGT
SEQ ID NO:36      (451)   AGTCCGTATAGGACTCTGATGAGTGTCAAAATAGGGCAATCACCTAATGT
SEQ ID NO:40      (451)   AGTCCGTATAGGACTTTGATGAGTGTCAAAATAGGGCAATCACCTAATGT
SEQ ID NO:42      (451)   AGTCCGTATAGGACTCTGATGAGTGTCAAAATAGGGCAATCACCTAATGT
                          501                                              550
SEQ ID NO:32      (501)   ATATCAAGCTAAATTTGAATCGGTGGCATGGTCAGCAACAGCATGCCATG
SEQ ID NO:36      (501)   ATATCAAGCTAAATTTGAATCGGTGGCATGGTCAGCAACAGCATGCCATG
SEQ ID NO:40      (501)   ATATCAAGCTAGATTTGAATCGGTGGCATGGTCAGCAACAGCATGCCATG
SEQ ID NO:42      (501)   ATATCAAGCTAAATTTGAATCGGTGGCATGGTCAGCAACAGCATGCCATG
                          551                                              600
SEQ ID NO:32      (551)   ATGGAAAAAAATGGATGACAGTTGGAGTCACAGGACCCGACAATCAAGCA
SEQ ID NO:36      (551)   ATGGAAAAAAATGGATGACAGTTGGAGTCACAGGACCCGACAATCAAGCA
SEQ ID NO:40      (551)   ATGGAAAAAAATGGATGACAGTTGGAGTCACAGGCCCCGACAATCAAGCA
SEQ ID NO:42      (551)   ATGGAAAAAAATGGATGACAGTTGGAGTCACAGGACCCGACAATCAAGCA
                          601                                              650
SEQ ID NO:32      (601)   ATTGCAGTAGTGAACTATGGAGGTGTTCCGGTTGATATTATTAATTCATG
SEQ ID NO:36      (601)   ATTGCAGTAGTGAACTATGGAGGTGTTCCGGTTGATATTATTAATTCATG
SEQ ID NO:40      (601)   ATTGCAGTAGTGAACTATGGAGGTGTTCCGGTTGATATTATTAATTCATG
SEQ ID NO:42      (601)   ATTGCAGTAGTGAACTATGGAGGTGTTCCGGTTGATATTATTAATTCATG
                          651                                              700
SEQ ID NO:32      (651)   GGCAGGGGATATTTTAAGAACCCAAGAATCATCATGCACCTGCATTAAAG
SEQ ID NO:36      (651)   GGCAGGGGATATTTTAAGAACCCAAGAATCATCATGCACCTGCATTAAAG
SEQ ID NO:40      (651)   GGCAGGGGATATTTTAAGAACCCAAGAGTCATCATGCACCTGCATTAAAG
SEQ ID NO:42      (651)   GGCAGGGGATATTTTAAGAACCCAAGAATCATCATGCACCTGCATTAAAG
                          701                                              750
SEQ ID NO:32      (701)   GAGACTGTTATTGGGTAATGACTGATGGACCGGCAAATAGGCAAGCTAAT
SEQ ID NO:36      (701)   GAGACTGTTATTGGGTAATGACTGATGGACCGGCAAATAGGCAAGCTAAT
SEQ ID NO:40      (701)   GAGACTGTTATTGGGTAATGACTGATGGACCGGCAAATAGGCAAGCTGAA
SEQ ID NO:42      (701)   GAGACTGTTATTGGGTAATGACTGATGGACCGGCAAATAGGCAAGCTAAT
                          751                                              800
SEQ ID NO:32      (751)   TATAGGATATTCAAAGCAAAAGATGGAAGAGTAATTGGACAAACTGATAT
SEQ ID NO:36      (751)   TATAGGATATTCAAAGCAAAAGATGGAAGAGTAATTGGACAAACTGATAT
SEQ ID NO:40      (751)   TATAGGATATTCAAAGCAAAAGATGGAAGAGTAATTGGGCAAACTGATAT
SEQ ID NO:42      (751)   TATAGGATATTCAAAGCAAAAGATGGAAGAGTAATTGGACAAACTGATAT
```

Figure 5N

```
                          801                                             850
SEQ ID NO:32     (801)    AAGTTTCAATGGGGGACACATAGAGGAGTGTTCTTGTTACCCCAATGAAG
SEQ ID NO:36     (801)    AAGTTTCAATGGGGGACACATAGAGGAGTGTTCTTGTTACCCCAATGAAG
SEQ ID NO:40     (801)    AAGTTTCAATGGGGGACACATAGAGGAGTGTTCTTGTTACCCCAATGAAG
SEQ ID NO:42     (801)    AAGTTTCAATGGGGGACACATAGAGGAGTGTTCTTGTTACCCCAATGAAG
                          851                                             900
SEQ ID NO:32     (851)    GGAAGGTGGAATGCATATGCAGAGACAATTGGACTGGAACAAAAAGACCA
SEQ ID NO:36     (851)    GGAAGGTGGAATGCATATGCAGAGACAATTGGACTGGAACAAAAAGACCA
SEQ ID NO:40     (851)    GGAAGGTGGAATGCATATGCAGGGACAATTGGACTGGAACAAATAGACCA
SEQ ID NO:42     (851)    GGAAGGTGGAATGCATATGCAGAGACAATTGGACTGGAACAAAAAGACCA
                          901                                             950
SEQ ID NO:32     (901)    ATTCTGGTAATATCTTCTGATCTATCGTACACAGTTGGATATTTGTGTGC
SEQ ID NO:36     (901)    ATTCTGGTAATATCTTCTGATCTATCGTACACAGTTGGATATTTGTGTGC
SEQ ID NO:40     (901)    ATTCTGGTAATATCTTCTGATCTATCGTACACAGTTGGATATTTGTGTGC
SEQ ID NO:42     (901)    ATTCTGGTAATATCTTCTGATCTATCGTACACAGTTGGATATTTGTGTGC
                          951                                            1000
SEQ ID NO:32     (951)    TGGCATTCCCACTGACACCCCTAGGGGAGAGGATAGTCAATTCACAGGCT
SEQ ID NO:36     (951)    TGGCATTCCCACTGACACCCCTAGGGGAGAGGATAGTCAATTCACAGGCT
SEQ ID NO:40     (951)    TGGCATTCCCACTGACACTCCTAGGGGAGAGGATAGTCAATTCACAGGCT
SEQ ID NO:42     (951)    TGGCATTCCCACTGACACCCCTAGGGGAGAGGATAGTCAATTCACAGGCT
                          1001                                           1050
SEQ ID NO:32    (1001)    CATGTACAAGTCCTTTGGGAAATAAAGGATACGGTGTGAAAGCTTTCGGG
SEQ ID NO:36    (1001)    CATGTACAAGTCCTTTGGGAAATAAAGGATACGGTGTGAAAGCTTTCGGG
SEQ ID NO:40    (1001)    CATGTACAAGTCCTTTGGGAAATAAGGGATACGGTGTAAAAGCCTTCGGG
SEQ ID NO:42    (1001)    CATGTACAAGTCCTTTGGGAAATAAAGGATACGGTGTGAAAGCTTTCGGG
                          1051                                           1100
SEQ ID NO:32    (1051)    TTTCGACAAGGAACTGACGTATGGCCGGAAGGACAATTAGTAGGACTTC
SEQ ID NO:36    (1051)    TTTCGACAAGGAACTGACGTATGGCCGGAAGGACAATTAGTAGGACTTC
SEQ ID NO:40    (1051)    TTTCGACAAGGAACTGACGTATGGCCGGAAGGACAATTAGTAGAACTTC
SEQ ID NO:42    (1051)    TTTCGACAAGGAACTGACGTATGGCCGGAAGGACAATTAGTAGGACTTC
                          1101                                           1150
SEQ ID NO:32    (1101)    AAGATCAGGATTCGAAATAATAAAAATCAGGAATGGCTGGACACAGAAAA
SEQ ID NO:36    (1101)    AAGATCAGGATTCGAAATAATAAAAATCAGGAATGGTTGGACACAGAAAA
SEQ ID NO:40    (1101)    AAGATCAGGATTCGAAATAATAAAAATCAGGAATGGTTGGACACAGAACA
SEQ ID NO:42    (1101)    AAGATCAGGATTCGAAATAATAAAAATCAGGAATGGTTGGACACAGAAAA
                          1151                                           1200
SEQ ID NO:32    (1151)    GTAAGGACCAAATCAGGAGGCAAGTGATTATCGATGACCTAAATTGGTCA
SEQ ID NO:36    (1151)    GTAAGGACCAAATCAGGAGGCAAGTGATTATCGATGACCCAAATTGGTCA
SEQ ID NO:40    (1151)    GTAAGGACCAAATCAGGAGGCAAGTGATTATCGATGACCCAAATTGGTCA
SEQ ID NO:42    (1151)    GTAAGGACCAAATCAGGAGGCAAGTGATTATCGATGACCCAAATTGGTCA
                          1201                                           1250
SEQ ID NO:32    (1201)    GGATATAGCGGTTCTTTCACATTGCCGGTTGAATTAACAAAAAAAGCATG
SEQ ID NO:36    (1201)    GGATATAGCGGTTCTTTCACATTGCCGGTTGAATTAACAAAAAAAGCATG
SEQ ID NO:40    (1201)    GGATATAGCGGTTCTTTCACATTGCCGGTTGAACTGACAAAAAAGGCATG
SEQ ID NO:42    (1201)    GGATATAGCGGTTCTTTCACATTGCCGGTTGAATTAACAAAAAAAGCATG
                          1251                                           1300
SEQ ID NO:32    (1251)    TTTGGTCCCCTGTTTCTGGGTTGAAATGATTAGAGGTAAACCTGAAGAAA
SEQ ID NO:36    (1251)    TTTGGTCCCCTGTTTCTGGGTTGAAATGATTAGAGGTAAACCTGAAGAAA
SEQ ID NO:40    (1251)    TTTGGTCCCCTGTTTCTGGGTTGAAATGATTAGAGGTAAACCTGAAGAAA
SEQ ID NO:42    (1251)    TTTGGTCCCCTGTTTCTGGGTTGAAATGATTAGAGGTAAACCTGAAGAAA
```

Figure 5O

```
                         1301                                              1350
SEQ ID NO:32  (1301)  CAACAATATGGACCTCTAGCAGCTCCATTGTGATGTGTGGAGTAGATCAT
SEQ ID NO:36  (1301)  CAACAATATGGACCTCTAGCAGCTCCATTGTGATGTGTGGAGTAGATCAT
SEQ ID NO:40  (1301)  CAACAATATGGACCTCTAGCAGCTCCATTGTGATGTGTGGAGTAGATCAT
SEQ ID NO:42  (1301)  CAACAATATGGACCTCTAGCAGCTCCATTGTGATGTGTGGAGTAGATCAT
                         1351                                              1400
SEQ ID NO:32  (1351)  AAAATTGCCAGTTGGTCATGGCACGATGGAGCAATTCTTCCCTTTGACAT
SEQ ID NO:36  (1351)  AAAATTGCCAGTTGGTCATGGCACGATGGAGCAATTCTTCCCTTTGACAT
SEQ ID NO:40  (1351)  AAAATTGCCAGTTGGTCATGGCACGATGGAGCTATTCTTCCCTTTGACAT
SEQ ID NO:42  (1351)  AAAATTGCCAGTTGGTCATGGCACGATGGAGCAATTCTTCCCTTTGACAT

                         1401
SEQ ID NO:32  (1401)  CGATAAGATG
SEQ ID NO:36  (1401)  CGATAAGATG
SEQ ID NO:40  (1401)  CGATAAGATG
SEQ ID NO:42  (1401)  CGATAAGATG
```

Sequence identity

SEQ ID NO:32 v. SEQ ID NO:36:	99.5%
SEQ ID NO:32 v. SEQ ID NO:40:	97.6%
SEQ ID NO:32 v. SEQ ID NO:42:	99.6%
SEQ ID NO:36 v. SEQ ID NO:40:	98.1%
SEQ ID NO:36 v. SEQ ID NO:42:	99.9%
SEQ ID NO:40 v. SEQ ID NO:42:	98.0%

Protein sequence alignment of NA protein of H3N8 and sequence identity

```
                         1                                                 50
SEQ ID NO:33     (1)  MNPNQKIIAIGSASLGILIINVILHVVSIIVTVLVLNNNRTDLNCKGTII
SEQ ID NO:37     (1)  MNPNQKIIAIGSASLGILIINVILHVVSIIVTVLVLNNNRTDLNCKGTII
SEQ ID NO:41     (1)  MNPNQKIIAIGFASLGILIINVILHVVSIIVTVLVLNNNRTDLNYKGTII
                         51                                               100
SEQ ID NO:33    (51)  KEYNETVRVEKLTQWYNNSTTKYIERPSNEYYMNNTEPLCEAQGFAPFSK
SEQ ID NO:37    (51)  KEYNETVRVEKLTQWYNNSTNKYIERPSNEYYMNNTEPLCEAQGFAPFSK
SEQ ID NO:41    (51)  KEYNETVKVEKLTQWYNTSTNKYIERPSNEYYMNNTEPLCEAQGFAPFSK
                         101                                              150
SEQ ID NO:33   (101)  DNGIRIGSRGHVFVIREPFVSCSPSECRTFFLTQGSLLNDKHSNGTIKDR
SEQ ID NO:37   (101)  DNGIRIGSRGHVFVIREPFVSCSPSECRTFFLTQGSLLNDKHSNGTIKDR
SEQ ID NO:41   (101)  DNGIRIGSRGHVFVIREPFVSCSPSECRTFFLTQGSLLNDKHSNGTIKDR
                         151                                              200
SEQ ID NO:33   (151)  SPYRTLMSVKIGQSPNVYQAKFESVAWSATACHDGKKWMTVGVTGPDNQA
SEQ ID NO:37   (151)  SPYKTLMSVKIGQSPNVYQAKFESVAWSATACHDGKKWMTVGVTGPDNQA
SEQ ID NO:41   (151)  SPYRTLMSVKIGQSPNVYQAKFESVAWSATACHDGKKWMTVGVTGPDNQA
                         201                                              250
SEQ ID NO:33   (201)  IAVVNYGGVPVDIINSWAGDILRTQESSCTCIKGDCYWVMTDGPANRQAK
SEQ ID NO:37   (201)  IAVVNYGGVPVDIINSWAGDILRTQESSCTCIKGDCYWVMTDGPANRQAK
SEQ ID NO:41   (201)  IAVVNYGGVPVDIINSWAGDILRTQESSCTCIKGDCYWVMTDGPANRQAE
```

Figure 5P

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                          251                                              300
SEQ ID NO:33   (251)  YRIFKAKDGRVIGQTDISFNGGHIEECSCYPNEGKVECICRDNWTGTNRP
SEQ ID NO:37   (251)  YRIFKAKDGRVIGQTDISFNGGHIEECSCYPNEGKVECICRDNWTGTNRP
SEQ ID NO:41   (251)  YRIFKAKDGRVIGQTDISFNGGHIEECSCYPNEGKVECICRDNWTGTNRP
                          301                                              350
SEQ ID NO:33   (301)  ILVISSDLSYTVGYLCAGIPTDTPRGEDSQFTGSCTSPLGNKGYGVKGFG
SEQ ID NO:37   (301)  ILVISSDLSYTVGYLCAGIPTDTPRGEDSQFTGSCTSPLGNKGYGVKGFG
SEQ ID NO:41   (301)  ILVISSDLSYTVGYLCAGIPTDTPRGEDSQFTGSCTSPLGNKGYGVKGFG
                          351                                              400
SEQ ID NO:33   (351)  FRQGTDVWAGRTISRTSRSGFEIIKIRNGWTQNSKDQIRRQVIIDDLNWS
SEQ ID NO:37   (351)  FRQGTDVWAGRTISRTSRSGFEIIKIRNGWTQNSKDQIRRQVIIDD[illegible]NWS
SEQ ID NO:41   (351)  FRQGTDVWAGRTISRTSRSGFEIIKIRNGWTQNSKDQIRRQVIIDD[illegible]NWS
                          401                                              450
SEQ ID NO:33   (401)  GYSGSFTLPVELTKKGCLVPCFWVEMIRGKPEETTIWTSSSSIVMCGVDH
SEQ ID NO:37   (401)  GYSGSFTLPVELTKKGCLVPCFWVEMIRGKPEETTIWTSSSSIVMCGVDH
SEQ ID NO:41   (401)  GYSGSFTLPVELTKKGCLVPCFWVEMIRGKPEETTIWTSSSSIVMCGVDH
                          451                470
SEQ ID NO:33   (451)  KIASWSWHDGAILPFDIDKM
SEQ ID NO:37   (451)  KIASWSWHDGAILPFDIDKM
SEQ ID NO:41   (451)  KIASWSWHDGAILPFDIDKM
```

Sequence identity
SEQ ID NO:33 v. SEQ ID NO:37: 99.6%
SEQ ID NO:33 v. SEQ ID NO:41: 98.3%
SEQ ID NO:37 v. SEQ ID NO:41: 98.7%

Sequence identity between NA proteins of H3N2 and H3N8

SEQ ID NO:	8	10	12	33	37	41
8		100%	100%	43.5%	43.8%	43.7%
10			100%	43.5%	43.8%	43.7%
12				44.1%	44.4%	44.3%
33					99.6%	98.3%
37						98.7%
41						

INACTIVATED CANINE INFLUENZA VACCINES AND METHODS OF MAKING AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application 62/185,266 filed on Jun. 26, 2015 and U.S. provisional application 62/298,285 filed on Feb. 22, 2016.

FIELD OF THE INVENTION

The present invention relates to new canine influenza virus strains, and vaccines and compositions. The present invention also relates to reagents and methods allowing their detection, methods of vaccination as well as methods of producing these reagents and vaccines.

BACKGROUND OF THE INVENTION

Influenza virus is a member of Orthomyxoviridae family (Murphy and Webster, Orthomyxoviruses, Fields Virology, Third Edition, vol. 1, pp. 1397-1445, 1996). There are three types of influenza viruses designated A, B, and C. The influenza virion contains a segmented negative-sense RNA genome. The influenza virion includes the following proteins: hemagglutinin (HA), neuraminidase (NA), matrix (M1), proton ion-channel protein (M2), nucleoprotein (NP), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), polymerase acidic protein (PA), and nonstructural protein 2 (NS2) proteins. The NP and the matrix protein M1 are used to classify the influenza virus into group A, B or C.

The HA and NA proteins are envelope glycoproteins. The HA protein is responsible for virus attachment and penetration of the viral particles into the cell and contains the major immunodominant epitopes for virus neutralization and protective immunity. Both HA and NA proteins are considered the most important components for prophylactic influenza vaccines. To date, eighteen different HA subtypes and eleven different NA subtypes have been identified (Tong et al., 2013, PLoS Pathogens, Vol. 9 (10), New World Bats harbor diverse influenza A viruses).

Globally, influenza is the most economically significant respiratory disease in humans, pigs, horses and poultry. Influenza virus is known for its continuous genetic and antigenic changes, which impede effective control of the virus. Of particular concern for prevention of epidemics and pandemics is the emergence of a new subtype of the virus by genetic re-assortment or inter-species transmission.

Recently, influenza outbreaks have occurred in species, e.g., feline and canine, which historically do not carry influenza virus. During 2004 and 2005, outbreaks of respiratory disease in racing greyhounds caused by infection with influenza virus occurred in Florida, eastern and western Iowa, and Texas. Molecular and antigenic analyses of three influenza viruses isolated from outbreaks of severe respiratory disease in racing greyhounds revealed that they are closely related to H3N8 equine influenza virus (Crawford et al., Science, 2005, 310 (5747):482-485; PLOS Pathogens, 2014, 10 (10), e1004455).

U.S. Pat. No. 8,246,962 and Song et al. (2008 Emerg. Infect. Dis., 2008, 14, pp. 741-746) reported an infection of an H3N2 subtype influenza virus in a pet dog in the Republic of Korea. The case was caused by a H3N2 avian-origin canine influenza virus (CIV), which infected dogs successfully through nasal inoculation or contact (respiratory fluid exchange) under experimental conditions.

Li et. al. (Infection, Genetics and Evolution, 2010, 10 (8): 1286-1288) reported four sporadic cases of H3N2 canine influenza in Southern China, which were identified from sick dogs from May 2006 to October 2007. The evolutionary analysis showed that all eight segments of these four viruses are avian-origin and phylogenetically closely related to the H3N2 canine influenza viruses reported earlier in South Korea.

H3N2 canine influenza can be transmitted to cats and cause severe respiratory disease in cats (Song et al., J. Gen. Virol. 2011, 92:23050-2355).

On Mar. 26, 2015, the Chicago Tribune reported an outbreak of canine influenza caused by a new strain of virus identified as H3N2. As of today, the virus has now been detected in dogs in 30 states, including Illinois, Ohio, Wisconsin, Indiana, Iowa, Minnesota, Michigan, California, Massachusetts, Texas, New Jersey, South Dakota, and Georgia.

There were no vaccines available in the U.S. for H3N2 CIV as of June 2015. The H3N8 CIV vaccines currently marketed in the U.S. are unlikely to protect dogs against H3N2 CIV due to the genetic divergence in the HA proteins encoded by the two virus subtypes. Although both viruses encode H3 subtype HAs, the proteins only share about 77% sequence identity at the amino acid level.

Accordingly, there is an urgent need for an effective vaccine against H3N2 influenza infection in canines and felines.

SUMMARY OF THE INVENTION

The present invention relates to an inactivated/killed canine influenza virus (CIV) composition or vaccine. In particular, the invention provides the canine influenza virus strains under the ATCC deposit Nos. PTA-122265 and PTA-122266, or any descendant or progeny of the strains. The canine influenza viruses are H3N2 strains isolated in the US.

In a particular embodiment, the inactivated vaccines comprise an adjuvant. The adjuvant may be any substance which increases and/or augments the elicited immune response, as compared to inactivated vaccine alone.

The invention also provides a polyvalent composition or vaccine that is effective to protect animals against a variety of CIV infections. In particular, the invention provides a polyvalent composition or vaccine comprising an inactivated H3N2 CIV and inactivated H3N8 CIV.

Methods for protection in animals against CIV infections and for reducing viral shedding in CIV infected animals are provided.

The invention also offers the possibility of diagnosing the presence of the canine influenza virus according to the invention in dogs. Its subject is therefore diagnostic tests and methods relating thereto using reagents which will be described below.

Kits comprising the inactivated canine Influenza strains and instructions for use are also provided.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, and which is not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference, in which:

FIGS. **1**A-B are tables showing the SEQ ID NO assigned to each DNA and protein sequence.

FIG. **2** depicts real-time RT-PCR genotyping of CIVs.

FIG. **3** depicts the sensitivity of the subtyping assay.

FIG. **4** depicts the efficiency of the subtyping assay.

FIG. **5**A-**5**P provide the sequence alignments of DNA and proteins.

DETAILED DESCRIPTION

It is noted that in this disclosure and particularly in the claims, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicate otherwise. The word "or" means any one member of a particular list and also includes any combination of members of that list.

The term "animal" is used herein to include all mammals, birds and fish. The animal as used herein may be selected from the group consisting of equine (e.g., horse), canine (e.g., dogs, wolves, foxes, coyotes, jackals), feline (e.g., lions, tigers, domestic cats, wild cats, other big cats, and other felines including cheetahs and lynx), bovine (e.g., cattle, buffalos), swine (e.g., pig), ovine (e.g., sheep), caprine (e.g., goats), camelids (e.g., lamas), avian (e.g., chicken, duck, goose, turkey, quail, pheasant, parrot, finches, hawk, crow, ostrich, emu and cassowary), primate (e.g., prosimian, tarsier, monkey, gibbon, ape), humans, and fish. The term "animal" also includes an individual animal in all stages of development, including embryonic and fetal stages.

The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of consecutive amino acid residues.

The term "nucleic acid", "nucleotide", and "polynucleotide" are used interchangeably and refer to RNA, DNA, cDNA, or cRNA and derivatives thereof, such as those containing modified backbones. It should be appreciated that the invention provides polynucleotides comprising sequences complementary to those described herein. The "polynucleotide" contemplated in the present invention includes both the forward strand (5' to 3') and reverse complementary strand (3' to 5'). Polynucleotides according to the invention can be prepared in different ways (e.g. by chemical synthesis, by gene cloning etc.) and can take various forms (e.g. linear or branched, single or double stranded, or a hybrid thereof, primers, probes etc.).

The term "genomic DNA" or "genome" is used interchangeably and refers to the heritable genetic information of a host organism. The genomic DNA comprises the DNA of the nucleus (also referred to as chromosomal DNA) but also the DNA of the plastids (e.g., chloroplasts) and other cellular organelles (e.g., mitochondria). The genomic DNA or genome contemplated in the present invention also refers to the RNA of a virus. The RNA may be a positive strand or a negative strand RNA. The term "genomic DNA" contemplated in the present invention includes the genomic DNA containing sequences complementary to those described herein. The term "genomic DNA" also refers to messenger RNA (mRNA), complementary DNA (cDNA), and complementary RNA (cRNA).

The term "gene" is used broadly to refer to any segment of polynucleotide associated with a biological function. Thus, genes or polynucleotides include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs, such as an open reading frame (ORF), starting from the start codon (methionine codon) and ending with a termination signal (stop codon). Genes and polynucleotides can also include regions that regulate their expression, such as transcription initiation, translation and transcription termination. Thus, also included are promoters and ribosome binding regions (in general these regulatory elements lie approximately between 60 and 250 nucleotides upstream of the start codon of the coding sequence or gene; transcription terminators (in general the terminator is located within approximately 50 nucleotides downstream of the stop codon of the coding sequence or gene). Gene or polynucleotide also refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

As used herein, the term "antigen" or "immunogen" means a substance that induces a specific immune response in a host animal. The antigen may comprise a whole organism, killed, attenuated or live; a subunit or portion of an organism; a recombinant vector containing an insert with immunogenic properties; a piece or fragment of DNA capable of inducing an immune response upon presentation to a host animal; a polypeptide, an antigen, an epitope, a hapten, or any combination thereof. Alternately, the immunogen or antigen may comprise a toxin or antitoxin.

The term "immunogenic protein or peptide" as used herein includes polypeptides that are immunologically active in the sense that once administered to the host, it is able to evoke an immune response of the humoral and/or cellular type directed against the protein. Preferably the protein fragment is such that it has substantially the same immunological activity as the total protein. Thus, a protein fragment according to the invention comprises or consists essentially of or consists of at least one epitope or antigenic determinant. An "immunogenic" protein or polypeptide, as used herein, includes the full-length sequence of the protein, analogs thereof, or immunogenic fragments thereof. By "immunogenic fragment" is meant a fragment of a protein which includes one or more epitopes and thus elicits the immunological response described above. Such fragments can be identified using any number of epitope mapping techniques, well known in the art. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance.

The term "immunogenic protein or peptide" further contemplates deletions, additions and substitutions to the sequence, so long as the polypeptide functions to produce an immunological response as defined herein. The term "conservative variation" denotes the replacement of an amino acid residue by another biologically similar residue, or the replacement of a nucleotide in a nucleic acid sequence such that the encoded amino acid residue does not change or is another biologically similar residue. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic—aspartate and glutamate; (2) basic—lysine, arginine, histidine; (3) non-polar—alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar—glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, or the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine, and the like; or a similar conservative replacement of an amino acid with a structurally related amino acid that will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the reference molecule but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the definition of the reference polypeptide. All of the polypeptides produced by these modifications are included herein. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

An "immunological response" to a composition or vaccine is the development in the host of a cellular and/or antibody-mediated immune response to a composition or vaccine of interest. Usually, an "immunological response" includes but is not limited to one or more of the following effects: the production of antibodies, B cells, helper T cells, and/or cytotoxic T cells, directed specifically to an antigen or antigens included in the composition or vaccine of interest. Preferably, the host will display either a therapeutic or protective immunological response such that resistance to new infection will be enhanced and/or the clinical severity of the disease reduced. Such protection will be demonstrated by either a reduction or lack of symptoms normally displayed by an infected host, a quicker recovery time and/or lowered pathogen loads in the infected host.

As used herein, the term "inactivated vaccine" means a vaccine composition containing an infectious organism or pathogen that is no longer capable of replication or growth. The pathogen may be bacterial, viral, protozoal or fungal in origin. Inactivation may be accomplished by a variety of methods including freeze-thawing, chemical treatment (for example, treatment with thimerosal, formalin, BPL (beta-propiolactone) or BEI (binary ethylenimine), sonication, radiation, heat or any other conventional means sufficient to prevent replication or growth of the organism while maintaining its immunogenicity.

The terms "polyvalent vaccine or composition", "combination or combo vaccine or composition" and "multivalent vaccine or composition" are used interchangeably to refer to a composition or vaccine containing more than one composition or vaccines. The polyvalent vaccine or composition may contain two, three, four or more compositions or vaccines. The polyvalent vaccine or composition may comprise recombinant viral vectors, active or attenuated or inactivated/killed wild-type viruses, or a mixture of recombinant viral vectors and wild-type viruses in active or attenuated or killed forms.

In one embodiment, the present invention encompasses a novel inactivated/killed CIV composition or vaccine. The CIV strains are the H3N2 subtype newly identified in the USA. The inactivation may be the chemical inactivation that produces enumerable structural changes, including for example, formation of new chemical bonds via chemical crosslinking, irreversible chemical alteration of the nucleic acid and protein coat.

In another embodiment, the present invention provides a bivalent composition or vaccine comprising an inactivated H3N2 CIV and inactivated H3N8 CIV.

One embodiment of the invention provides the genomic DNA and gene sequences, and encoded protein sequences of CIV H3N2 and H3N8 strains.

In another embodiment, the invention provides the sequences for HA proteins or antigens. In one aspect of the embodiment, the HA proteins have the polypeptide sequence as set forth in SEQ ID NO:2, 4, 6, 31, 35, and 39. In another aspect, the HA proteins have at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%. 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:2, 4, 6, 31, 35, and 39. In yet another aspect, the HA proteins are encoded by the polynucleotides having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%. 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:1, 3, 5, 30, 34 and 38.

In another embodiment, the invention provides the sequences for NA proteins or antigens. In one aspect of the embodiment, the NA proteins have the polypeptide sequence as set forth in SEQ ID NO:8, 10, 12, 33, 37 and 41. In another aspect, the NA proteins have at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%. 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to a polypeptide having the sequence as set forth in SEQ ID NO:8, 10, 12, 33, 37 and 41. In yet another aspect, the NA proteins are encoded by the polynucleotides having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.1%. 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8% or 99.9% sequence identity to a polynucleotide having the sequence as set forth in SEQ ID NO:7, 9, 11, 32, 36, 40 and 42.

In one embodiment, the CIV stains may comprise an HA gene encoding an HA protein aforementioned. In another embodiment, the CIV strains may comprise an NA gene encoding an NA protein aforementioned.

In one embodiment, the invention provides CIV strains under the ATCC deposit numbers PTA-122265 and PTA-122266, or any parent, descendant or progeny of the deposited strains.

In one embodiment, the composition or vaccine of the invention includes a live CIV H3N2 strain. The CIV H3N2 strains were initially isolated from 1-14 year old dogs with symptoms of severe respiratory disorder in Illinois, USA. The strains were deposited at ATCC (American Type Culture Collection; 10801 University Boulevard, Manassas, VA 20110) on Jun. 25, 2015 and were accorded Accession Nos. PTA-122265 and PTA-122266.

In another embodiment, the present invention contemplates preparation and isolation of a progeny or descendant of a CIV H3N2 virus that has been deposited on Jun. 25, 2015 at ATCC under the Accession Numbers PTA-122265 and PTA-122266. The invention therefore extends to CIV H3N2 virus strains which are derived from the deposited strains through propagation or multiplication in an identical or divergent form, in particular descendants which possess the essential characteristics of the deposited strains. Upon continued propagation, the strains may acquire mutations most of which will not alter the properties of these strains significantly. The progeny or descendant may comprise a polynucleotide encoding an HA protein having at least 99.6% sequence identity to the sequence as set forth in SEQ ID NO:2, 4, or 6, or a polynucleotide encoding an NA protein having the sequence as set forth in SEQ ID NO:8, 10 or 12. The progeny or descendant may comprise a polynucleotide encoding an HA protein having at least 99.0% or 99.6% sequence identity to the sequence as set forth in SEQ ID NO:2, 4, or 6 and the HA protein may differ at amino acid position 62, 219 or 249 of a full-length HA0 protein (i.e. uncleaved and containing the signal peptide). The amino acid at position 62, 219, or 249 of a full-length HA0 protein may be any amino acid denoted in Table 1 below.

TABLE 1

Amino Acid			
Abbreviation	Amino Acid	Abbreviation	Amino Acid
A (Ala)	Alanine	L (Leu)	Leucine
R (Arg)	Arginine	K (Lys)	Lysine
D (Asp)	Aspartic acid	M (Met)	Methionine
N (Asn)	Asparagine	F (Phe)	Phenylalanine
C (Cys)	Cysteine	P (Pro)	Proline
Q (Gln)	Glutamine	S (Ser)	Serine
E (Glu)	Glutamic acid	T (Thr)	Threonine
G (Gly)	Glycine	W (Trp)	Tryptophan
H (His)	Histidine	Y (Tyr)	Tyrosine
I (Ile)	Isoleucine	V (Val)	Valine

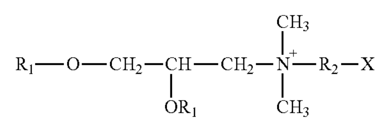
The inactivated pathogen or organism can be concentrated by conventional concentration techniques, in particular by ultrafiltration, and/or purified by conventional purification means, in particular using chromatography techniques including, but not limited to, gel-filtration or by ultrafiltration. As used herein, the term "immunogenicity" means capable of producing an immune response in a host animal against an antigen or antigens. This immune response forms the basis of the protective immunity elicited by a vaccine against a specific infectious organism.

Further, methods which are well known to those skilled in the art can be used to determine protein purity or homogeneity, such as polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polypeptide band on a staining gel. Higher resolution may be determined using HPLC or other similar methods well known in the art.

In one embodiment, the invention provides for the administration of a therapeutically effective amount of a vaccine or composition for the delivery and expression of a CIV H3N2 antigen in a target cell. In another embodiment, the invention provides for the administration of a therapeutically effective amount of a vaccine or composition for the delivery and expression of CIV H3N2 and CIV H3N8 antigens in a target cell. Determination of the therapeutically effective amount is routine experimentation for one of ordinary skill in the art. In one embodiment, the vaccine or composition comprises an inactivated/killed CIV H3N2 strains, and a pharmaceutically or veterinarily acceptable carrier, adjuvant, vehicle or excipient. In another embodiment, the vaccine or composition comprises an inactivated/killed H3N2 CIV and inactivated/killed H3N8 CIV strains, and a pharmaceutically or veterinarily acceptable carrier, adjuvant, vehicle or excipient. In an embodiment, the pharmaceutically or veterinarily acceptable carrier, vehicle, adjuvant, or excipient facilitates transfection and/or improves preservation of the virus or protein.

The pharmaceutically or veterinarily acceptable carriers, adjuvants, vehicles, or excipients are well known to the one skilled in the art. For example, a pharmaceutically or veterinarily acceptable carrier or vehicle or excipient can be a 0.9% NaCl (e.g., saline) solution or a phosphate buffer. Other pharmaceutically or veterinarily acceptable carrier, adjuvant, vehicle, or excipients that can be used for methods of this invention include, but are not limited to, poly-(L-glutamate) or polyvinylpyrrolidone. The pharmaceutically or veterinarily acceptable carrier, adjuvant, vehicle, or excipients may be any compound or combination of compounds facilitating the administration of the vector (or protein expressed from an inventive vector in vitro); the carrier, vehicle, adjuvant, or excipient may facilitate transfection and/or improve preservation of the vector (or protein). Doses and dose volumes are herein discussed in the general description and can also be determined by the skilled artisan from this disclosure read in conjunction with the knowledge in the art, without any undue experimentation.

The cationic lipids containing a quaternary ammonium salt which are advantageously but not exclusively suitable for plasmids, are advantageously those having the following formula:



in which R_1 is a saturated or unsaturated straight-chain aliphatic radical having 12 to 18 carbon atoms, R_2 is another aliphatic radical containing 2 or 3 carbon atoms and X is an amine or hydroxyl group, e.g. the DMRIE. In another embodiment the cationic lipid can be associated with a neutral lipid, e.g. the DOPE.

Among these cationic lipids, preference is given to DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propane ammonium; WO96/34109), advantageously associated with a neutral lipid, advantageously DOPE (dioleoyl-phosphatidyl-ethanol amine; Behr J. P., 1994, Bioconjugate Chemistry, 5, 382-389), to form DMRIE-DOPE.

The composition or vaccine mixture with the adjuvant is formed extemporaneously and contemporaneously with administration of the preparation or shortly before administration of the preparation; for instance, shortly before or prior to administration, the composition or vaccine-adjuvant mixture is formed, so as to give enough time prior to administration for the mixture to form a complex, e.g. between about 10 and about 60 minutes prior to administration, such as approximately 30 minutes prior to administration.

When DOPE is present, the DMRIE:DOPE molar ratio is advantageously about 95:about 5 to about 5:about 95, more advantageously about 1:about 1, e.g., 1:1.

The immunogenic compositions and vaccines according to the invention may comprise or consist essentially of one or more adjuvants. Suitable adjuvants for use in the practice of the present invention are (1) polymers of acrylic or methacrylic acid, maleic anhydride and alkenyl derivative polymers, (2) immunostimulating sequences (ISS), such as oligodeoxyribonucleotide sequences having one ore more non-methylated CpG units (Klinman D. M. et al., Proc. Natl. Acad. Sci., USA, 1996, 93, 2879-2883; WO98/16247), (3) an oil in water emulsion, such as the SPT emulsion described on p 147 of "Vaccine Design, The Subunit and Adjuvant Approach" published by M. Powell, M. Newman, Plenum Press 1995, and the emulsion MF59 described on p 183 of the same work, (4) cation lipids containing a quaternary ammonium salt, (5) cytokines, (6) aluminum hydroxide or aluminum phosphate or (7) saponin, (8) Dimethyldioctadecyl ammonium bromide (Vaccine Design p. 157), (9) Aridine (Vaccine Design p. 148) other adjuvants discussed in any document cited and incorporated by reference into the instant application, or (8) any combinations or mixtures thereof.

The oil in water emulsion (3), can be based on: light liquid paraffin oil (European pharmacopoeia type), isoprenoid oil such as squalane, squalene, oil resulting from the oligomerization of alkenes, e.g. isobutene or decene, esters of acids or alcohols having a straight-chain alkyl group, such as vegetable oils, ethyl oleate, propylene glycol, di(caprylate/caprate), glycerol tri(caprylate/caprate) and propylene glycol dioleate, or esters of branched, fatty alcohols or acids, especially isostearic acid esters.

The oil is used in combination with emulsifiers to form an emulsion. The emulsifiers may be nonionic surfactants, such as: esters of on the one hand sorbitan, mannide (e.g. anhydromannitol oleate), glycerol, polyglycerol or propylene glycol and on the other hand oleic, isostearic, ricinoleic or hydroxystearic acids, said esters being optionally ethoxylated, or polyoxypropylene-polyoxyethylene copolymer blocks, such as Pluronic, e.g., L121. Some of the emulsions, such as TS6, TS7, TS8 and TS9 emulsions, are described in U.S. Pat. Nos. 7,608,279 and 7,371,395.

The polymers of acrylic or methacrylic acid (1) are preferably crosslinked, in particular with polyalkenyl ethers of sugars or polyalcohols. These compounds are known under the term carbomer (Pharmeuropa vol. 8, No. 2, June 1996). Persons skilled in the art can also refer to U.S. Pat. No. 2,909,462 describing such acrylic polymers crosslinked with a polyhydroxylated compound having at least 3 hydroxyl groups, preferably not more than 8, the hydrogen atoms of at least three hydroxyls being replaced with unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing 2 to 4 carbon atoms, e.g. vinyls, allyls and other ethylenically unsaturated groups. The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name CARBOPOL™ (BF Goodrich, Ohio, USA) are particularly appropriate. They are crosslinked with an allyl sucrose or with allylpentaerythritol. Among them, there may be mentioned CARBOPOL™ 974P, 934P and 971 P.

Among the copolymers of maleic anhydride and of alkenyl derivative, the EMA™ copolymers (Monsanto) which are copolymers of maleic anhydride and of ethylene, which are linear or crosslinked, for example crosslinked with divinyl ether, are preferred.

The proportions of adjuvant which are useful are well known and readily available to the one skilled in the art. By way of example, the concentration of polymers of acrylic or methacrylic acid or of anhydride maleic and alkenyl copolymers in the final vaccine composition will be from 0.01% to 1.5% W/V, more particularly from 0.05 to 1% W/V, preferably from 0.1 to 0.4% W/V.

In one embodiment, the adjuvant may include TS6 (U.S. Pat. No. 7,371,395), LR2, LR3 and LR4 (U.S. Pat. No. 7,691,368), TSAP (US20110129494), TRIGEN™ (Newport Labs), synthetic dsRNAs (e.g. poly-IC, poly-ICLC [HILTONOL®]), and MONTANIDE™ adjuvants (W/O, W/O/W, O/W, IMS and Gel; all produced by SEPPIC).

In yet another embodiment, the adjuvant may include interleukin-2 (IL-2), IL-12, interferon α (IFNα), polyinosinic and polycytidylic acid, and cytidine-phosphate-guanosine oligodeoxynucleotides (CpG ODN), which are known to significantly enhance CMI response to CIV vaccines (Vet. Immuno. and Immunopath. Vol. 129, Issues 1-2, 15 May 2009, Pages 1-13).

Optionally the vaccine used according to the method of the invention may contain a cytokine. The cytokine may be present as a protein or as a gene encoding this cytokine inserted into a recombinant viral vector. The cytokines may be selected among the pig cytokines.

In a specific embodiment, the pharmaceutical composition is directly administered in vivo. Advantageously, the pharmaceutical and/or therapeutic compositions and/or formulations according to the invention comprise or consist essentially of or consist of an effective quantity to elicit a therapeutic response of one or more expression vectors and/or polypeptides as discussed herein; and, an effective quantity can be determined from this disclosure, including the documents incorporated herein, and the knowledge in the art, without undue experimentation.

The composition or vaccine may contain a dose from about 10^2 to about 10^{20}, about 10^3 to about 10^{18}, about 10^4 to about 10^{16}, about 10^5 to about 10^{12} VLPs (virus like particles) produced in vitro or in vivo from a viral vector, a plasmid, or baculovirus. The viral vector may be titrated based on any virus titration methods including, but not limited to, FFA (Focus Forming Assay) or FFU (Focus Forming Unit), $TCID_{50}$ (50% Tissue Culture Infective Dose), PFU (Plaque Forming Units), and $FAID_{50}$ (50% Fluorescent Antibody Infectious Dose), and the VLPs produced in vitro can be titrated by hemagglutination assay, ELISA, and electron microscopy. Other methods may also be applicable depending on the type of VLP.

The composition or vaccine may contain from about $10^{2.0}$ to about $10^{10.0}$ $TCID_{50}$/dose, from about $10^{4.5}$ to about $10^{8.0}$ $TCID_{50}$/dose and from about $10^{5.5}$ to about $10^{6.5}$ $TCID_{50}$/dose. The composition or vaccine may contain equivalent $TCID_{50}$ in the case of inactivated/killed composition or vaccine.

The dose volumes can be between about 0.1 and about 10 ml, between about 0.2 and about 5 ml.

When the antigen relates to hemagglutinin, such as inactivated influenza vaccines, the dosage is measured in hemagglutination units (HAUs). In an embodiment, the dosage may be from about 655 to about 65,500 HAU/dose.

It should be understood by one of skill in the art that the disclosure herein is provided by way of example and the present invention is not limited thereto. From the disclosure herein and the knowledge in the art, the skilled artisan can determine the number of administrations, the administration route, and the doses to be used for each injection protocol, without any undue experimentation.

The present invention contemplates at least one administration to an animal of an efficient amount of the therapeutic composition made according to the invention. The animal may be male, female, pregnant female and newborn. This administration may be via various routes including, but not limited to, intramuscular (IM), intradermal (ID) or subcutaneous (SC) injection or via intranasal or oral administration. The therapeutic composition according to the invention can also be administered by a needleless apparatus (as, for example with a Pigjet, Biojector or Vitaj et apparatus (Bioject, Oreg., USA)).

The composition or vaccine is administered to a dog or a cat (about six to eight-week old). A booster administration can be done if necessary around 2 to 8 weeks after the first administration.

Liquid jet needle-free injectors are devices performing injections of a certain amount of liquid under high pressure through a minute orifice. In an embodiment, the needle-free injection is a DERMA-VAC NF transdermal vaccinator system.

The volume of dose injected may be from about 0.1 ml to about 4.0 ml, about 0.1 ml to about 2 ml, about 0.1 ml to about 1 ml, from about 0.2 ml to about 0.8 ml, about 0.2 ml to about 0.5 ml. By definition, the volume of one dose means the total volume of vaccine administered at once to one animal.

Optionally, the administration can be repeated, as booster administration, at suitable intervals if necessary or desirable, e.g. from about 2 to about 8 weeks after the first administration, and preferably from about 2 to about 4 weeks after the first administration. A booster administration can also be repeated every 6 months or every year.

Another object of the invention is the use of an efficient amount of the composition or vaccine as described above and of an acceptable vehicle or diluent, for the preparation of a vaccine designed to be administered to an animal using a liquid jet needle-free injector as described above, and resulting in eliciting a safe and protective immune response against influenza infection.

In one embodiment of the invention, a prime-boost regimen can be employed, which is comprised of at least one primary administration and at least one booster administration using at least one common polypeptide, antigen, epitope or immunogen. Typically the same composition or vaccine is used as the primary administration and the boost. This administration protocol is called "prime-boost". However, different compositions or vaccines may be used as the prime administration and the boost.

Another object is a vaccination kit or set, comprising at least one vaccine vial containing the vaccine of the present invention, and operatively assembled to perform the administration of the vaccine to an animal of the canine family. Such vaccination kit or set is able to elicit a safe and protective immune response against influenza infection.

The invention also offers the possibility of diagnosing the presence of the CIV H3N2 or H3N8 strains according to the invention in dogs and cats. Its subject is therefore diagnostic tests and methods relating thereto using reagents which are described below.

A first reagent relates to the DNA sequences disclosed here and their fragments, which may be used as probes or primers in well-known hybridization or PCR (Polymerase Chain Reaction) techniques.

A second reagent relates to the polypeptides encoded by these sequences from the virus, or synthesized by the chemical route according to conventional techniques for peptide synthesis.

A third and fourth reagent relate to polyclonal and monoclonal antibodies which may be produced according to the customary techniques from the virus, the polypeptides or fragments, extracted or encoded by the DNA sequences.

These second, third and fourth reagents may be used in a diagnostic method, a subject of the invention, in which a test is carried out, on a sample of physiological fluid (blood, plasma, serum and the like) or a sample of tissue (ganglia, liver, lungs, kidneys and the like) obtained from a dog or a cat to be tested, for the presence of an antigen specific for a CIV H3N2 or H3N8 according to the invention, by seeking to detect either the antigen itself, or antibodies directed against this antigen.

The antigens and antibodies according to the invention may be used in any known laboratory diagnostic technique. However, it will be preferable to use them in techniques which can be used directly in the field by the veterinary doctor, the breeder or the owner of the animal. Persons skilled in the art have available a range of laboratory and field techniques and are therefore in the perfect position to adapt the use of this antigen and/or antibodies as diagnostic reagent(s).

The diagnostic techniques which will be used within the framework of the present invention are PCR and RT-PCR.

The subject of the invention is also a diagnostic kit comprising the reagents and/or polyclonal or monoclonal antibodies specific for H3N2 or H3N8 CIV antigen. The diagnostic kit may comprise primers designed based on the DNA sequences disclosed herein and reagents for PCR assay.

The method of diagnosing the presence of a CIV H3N2 or H3N8 strain using PCR comprises the steps of: a) generating a cDNA of the influenza virus genome isolated from an animal; b) exposing the cDNA to a primer pair comprising a forward primer and a reverse primer in a real-time polymerase chain reaction (PCR) to yield an amplicon, wherein the primer pair is specific to the H3N2 or H3N8 CIV genome; c) performing sequencing on the amplicon; and d) analyzing and comparing the sequence of the amplicon with known H3N2 or H3N8 CIV sequences.

The method of diagnosing the presence of an H3N2 or H3N8 CIV strain using real-time RT-PCR comprises the steps of: a) extracting genomic RNA from the influenza virus isolated from an animal; b) exposing the RNA to a primer pair comprising a forward and a reverse primer in a real-time reverse transcription-polymerase chain reaction (RT-PCR), wherein the primer pair is specific to the H3N2 or H3N8 CIV genome; c) analyzing and comparing the RT-PCR curve thereby characterizing the CIV strain.

The forward primer and reverse primer may be designed based on the HA and NA polynucleotide sequences disclosed herein. The primers may be the primers having the sequence as defined in SEQ ID NO:13-29 (FIG. **1** and Table 3).

The diagnostic techniques which will be used within the framework of the present invention include Western blotting, immunofluoroescence, ELISA and immunochromatography.

Accordingly, it is preferably sought to detect specific antibodies in the sample by an indirect test, by competition or by displacement. To do this, the antigen itself is used as diagnostic reagent, or a fragment of this antigen, conserving recognition of the antibodies. The labelling may be advantageously a labelling with peroxidase or a special labelling, preferably with colloidal gold.

It may also be desired to detect the antigen itself in the sample with the aid of a labelled antibody specific for this antigen. The labelling is advantageously as described above.

By antibody specific for the antigen which can be used in particular in competition or displacement or for the detection of the antigen itself, there is understood monoclonal or polyclonal antibodies specific for the antigen, fragments of these antibodies.

Another feature of the invention is the production of polyclonal or monoclonal antibodies specific for the antigen in accordance with the invention, it being possible for these antibodies to then be used in particular as diagnostic reagent for the detection of the antigen in a sample of physiological fluid or in a tissue sample, or even for the detection of antibodies present in such a sample or specimen. The invention also includes the immunologically functional fragments of these antibodies.

Antibodies can be prepared by the customary techniques. Reference may be made in particular to Antibodies, A Laboratory Manual, 2014, Cold Spring Harbor Laboratory, USA.

The subject of the invention is also a preparation, pure or partially pure, or even crude, of monoclonal or polyclonal antibodies specific for the antigen, especially mouse or rabbit antibodies.

The invention will now be further described by way of the following non-limiting examples.

EXAMPLES

Construction of DNA inserts, plasmids and recombinant viral vectors was carried out using the standard molecular biology techniques described by J. Sambrook et al. (Molecular Cloning: A Laboratory Manual, 4th edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2014).

Example 1 Culture, Isolation and Genotyping of Canine Influenza H3N2 Strains

Virus Isolation

Nasal swabs were obtained from pet dogs that exhibited symptoms of acute respiratory disease at veterinary clinics in Chicago, Ill. Swabs were transferred to vials containing transport Minimal essential medium (MEM) supplemented with 2% fetal bovine serum (HyClone) and 1× Antibiotic-Antimycotic (Gibco). Vials were placed at 4° C. and shipped cold by overnight courier within 4 days.

Infectious virus was isolated from clinical samples as follows. Samples were vortexed, and swabs were pressed against the vial walls to remove absorbed medium. The medium in each vial was filtered through a 0.4 μM filter, and 100 μL of filtered medium was inoculated into Madin-Darby Canine Kidney (MDCK) cells (ATCC) or 10 day old specific pathogen free (SPF) embryonated chicken eggs (Merial, Inc., Gainesville). Cell cultures were incubated at 37° C., and cell supernatants were harvested when a majority of cells exhibited virus-induced cytopathic effect (CPE). Inoculated eggs were incubated at 37° C. for 72 hours and subsequently chilled at 4° C. for at least 6 hours. To identify eggs that contained influenza virus, allantoic fluid from each inoculated egg was subjected to hemagglutination (HA) assay using 0.5% chicken red blood cells (RBCs) (Merial, Inc., Gainesville). Eggs containing >2 HA units per 50 μL of allantoic fluid were considered HA-positive, and the full volume of allantoic fluid in such eggs was removed, aliquoted, and frozen at −70° C. Five of twenty-four samples inoculated into eggs showed positive HA titers with values ranging from 32-256 HA units/50 μL. Two of the five samples (virus isolates ID #'s 4 and 8) were chosen for further vaccine development purposes and deposited at ATCC on Jun. 25, 2015 under accession number PTA-122265 (virus ID #4) and PTA-122266 (virus ID #8).

Viral RNA detection and virus subtyping

A real-time RT-PCR assay was developed to test for the presence and subtype of influenza virus in egg allantoic fluid. Primers pairs that specifically anneal to the N2 or N8 neuraminidase (NA) segment genomic RNA encoded by H3N2 and H3N8 subtype canine influenza viruses (CIV) were designed based on sequence alignments of NA gene sequences from CIVs available in GenBank. Forward primer SEQ ID NO:13 and reverse primer SEQ ID NO:14 were specific for the N2 NA gene of H3N2 CIV. Forward primer SEQ ID NO:15 and reverse primer SEQ ID NO:16 were specific for the N8 NA gene of H3N8 CIV.

RNA extracted from 140 μL of egg allantoic fluid (QIAamp Viral RNA Mini Kit; Qiagen) was tested for the presence of N2 and N8 genomic RNA using a QuantiTect SYBR Green RT-PCR Kit (Qiagen). Assay results (see FIG. **2** and Table 2) showed that the N2-specific primers produced amplification products with cycle threshold (C_t) values of 9.1-35, while the N8 specific primer pairs produced products with C_t values >39 (the limit of detection). Conversely, RNA from eggs inoculated with a H3N8 subtype CIV (A/canine/Wyoming/86033/2007) yielded a product with a C_t value of 15.4 when tested with N8-specific primers, but N2-specific primers did not yield detectable products. Thus, the virus isolated from the nasal swabs contained N2 but not N8 NA genomic RNA, suggesting that the isolated virus was H3N2 CIV.

TABLE 2

RT-PCR genotyping of CIVs

RNA source	Virus subtype	Primer target	C_t
None	N/A	N2 NA	ND
None	N/A	N8 NA	ND
A/Ca/WY/86033/07	H3N8	N2 NA	ND
A/Ca/WY/86033/07	H3N8	N8 NA	15.4
#4	H3N2	N2 NA	9.1
#4	H3N2	N8 NA	39.1
#8	H3N2	N2 NA	9.5
#8	H3N2	N8 NA	ND

*ND—not detected.

FIGS. **3** and **4** show the sensitivity and efficiency of the subtyping assay. The assay is highly sensitive for H3N2 CIV as it is capable of detecting <0.01 EID50 units of virus (i.e. it can detect less than one infectious unit of virus). The assay is also highly efficient. FIG. **4** shows that the assay is 98.81% efficient on a scale from 1-100%. The amplification factor of the assay is 1.99, close to the perfect score of 2.0 (amplification factors are calculated in such a way that a value of 2.0 is considered perfect).

Example 2 Virus Propagation and Genomic RNA

Virus propagation in eggs

The 50% egg infectious dose (EID_{50}) assay was used to determine the titer of infectious virus present in the virus isolates. Virus samples were diluted in 10-fold increments from 10^{-1} to 10^{-9}, and five eggs per dilution were inoculated with 100 μL per egg of the 10^{-4} to 10^{-9} dilutions. Eggs were sealed and incubated for 72 hours at 37° C. Eggs were then chilled at 4° C. for at least 6 hours, and allantoic fluid from each egg was tested for the presence of virus by HA assay. The HA titers ranged from 64-256 per 50 μL of allantoic fluid for viruses #4 and #8, and the EID_{50}/mL titer was 8.3 (#4) and 8.1 (#8) as calculated using the Spearman-Karber formula.

Large-scale virus stocks were prepared by inoculating each of sixty eggs with 100 μL of a 10^{-5} virus dilution. Eggs were incubated at 37° C. for 72 hours and chilled as before. Allantoic fluid was collected from each egg and pooled. Virus HA and EID_{50} titers in the pooled fluids were 16 and 7.5 (#4) or 8 and 7.3 (#8). Virus stocks were aliquoted and stored in liquid nitrogen.

Virus Propagation in Madin-Darby Canine Kidney (MDCK) Cells

MDCK cells (ATCC) approved for vaccine derivation purposes by the USDA were seeded such that cell monolayers were ~90% confluent at the time of infection. Prior to infection, cells were washed twice with MEM lacking serum and inoculated with virus using a multiplicity of infection (M.O.I.) of 0.001 in a low inoculum volume. Samples were incubated at 37° C. for 1 hour with intermittent rocking, and media containing 1 ug/mL of TPCK-treated trypsin (Sigma-Aldrich) was added. Cultures were incubated at 37° C. and monitored for the appearance of virus-induced cytopathic effect (CPE). Under these conditions, the virus caused visible cell rounding and dissociation of cells from the substrate after 24-hours. Cell supernatants were harvested when approximately 90% of the cells had dissociated from the substrate. Supernatants were clarified by centrifugation at 1,000×g, and virus titers in the clarified supernatants were measured by HA and 50% tissue culture infectious dose ($TCID_{50}$) assays. After a single passage in MDCK cells, virus #4 achieved titers of 64 HA units/50 μL and 7.8 $TCID_{50}$ units/mL, and virus #8 replicated to titers of 64-128 HA units/50 μL and 8.6 $TCID_{50}$ units/mL. These cell stocks were subsequently used to produce pre-master seed stock material (virus #4, 64-128 HA units/50 μL and 8.4 $TCID_{50}$ units/mL) and purified antigen for raising virus-specific antisera in rabbits (virus #8, 128 HA units/50 μL and 8.7 $TCID_{50}$ units/mL).

Viral genomic RNA sequencing

Genomic RNA was extracted from virus propagated in eggs and MDCK cells. The full-length HA and NA genes were reverse transcribed and amplified by PCR using a One-Step RT-PCR Kit (Qiagen) and universal primers that anneal to the termini of each gene segment (Hoffman et al., 2001 Arch Virol Universal primer set for the full-length amplification of all influenza A viruses). The resulting RT-PCR products were separated by electrophoresis in a 0.8% agarose gel and purified using a QIAquick Gel Extraction Kit (Qiagen). Purified cDNAs were sequenced by conventional Sanger sequencing methods using HA and NA gene specific primers as shown in Table 3 below. Contiguous sequence assemblies with 2-fold or greater coverage were generated by aligning overlapping individual sequences using Sequencher 5.1 software (Gene Codes Corporation). Consensus sequences for each gene were generated from the assemblies in Sequencher 5.1 and designated SEQ ID NOs: 1, 3, 5, 7, 9 and 11 as shown in FIGS. **1** and **5**. The corresponding HA and NA protein sequences are designated SEQ ID NOs:2, 4, 6, 8, 10 and 12 as shown in FIGS. **1** and **5**.

TABLE 3

primers for sequencing HA and NA genes

SEQ ID NO:		
	HA sequencing primers	
17	Ca.H3N2.HA.390R	GAACTCCAATGTGCCTGATG
18	Ca.H3N2.HA.259F	CTGCACACTAATAGATGCCCTA
19	Ca.H3N2.HA.743F	CCAATCTGGCAGAATAAGCG
20	Ca.H3N2.HA.1276F	GAAGGGAGGATTCAAGACCTT
21	Ca.H3N2.HA.1569F	GGTTCCAGATCAAAGGTGTT
22	Ca.H3N2.HA.871R	CATTCGGAAATGCAGGTGTCA
23	Ca.H3N2.HA.1408R	TCAGCATTTTCCCTCAATTGC
	NA gene sequencing primers	
24	Ca.H3N2.NA.429F	GGG ACC ACG CTG AAC AAT AA
25	Ca.H3N2.NA.484R	TGA AAC GGA ACA CCC AAC TC
26	Ca.H3N2.NA.975F	TCA GGA CTT GTT GGC GAT AC
27	Ca.H3N2.NA.1023R	TCC TGG ATT CCC TCT CTC ATT A
28	Ca.H3N2.1229F	ACT GGT CTG GTT ATT CTG GTA TTT
29	Ca.H3N2.1279R	TCT TGT GGC CCT CCT CTT AT

Analysis of the Sequences

The three HA protein sequences obtained from the CIV H3N2 strains isolated in the US share 99.6% sequence identity (FIG. **5**). These HA proteins vary in the amino acid composition at positions 62, 219 and 249 of the immature (full-length) HA0 protein.

The three NA protein sequences obtained from the CIV H3N2 strains isolated in the US are identical, though the polynucleotides encoding the NA proteins differ slightly with 99.9% sequence identity (FIG. **5**). The database search didn't detect any CIV H3N2 NA proteins having greater than 98% sequence identity to the NA protein sequences generated from the CIV H3N2 strains isolated in the US.

Example 3 Production of Virus-Specific Antibodies

Virus purification

MDCK cells in 225 cm^2 flasks were washed twice with 20 mL MEM per flask and inoculated with cell passage 1 of virus #8 at an MOI of 0.001 in a low inoculum volume. Cultures were incubated at 37° C. for 1 hour with intermittent rocking. Medium containing 1 ug/mL of TPCK-treated trypsin was added to each flask, and the flasks were incubated at 37° C. for 48 hours. Cell supernatants were harvested, and 540 mL of virus culture was clarified by centrifugation at 5,000×g for 10 min. using a Beckman JA-10 rotor. To pellet the virus, the clarified supernatant was centrifuged at 90,000×g for 2 hours at 4° C. using a SW32 Ti rotor. The pellet supernatant was aspirated, and 0.5 mL PBS was added to each virus pellet. Following overnight incubation on ice, the pellets were resuspended by gentle pipetting and pooled. The resuspended pellet material was loaded onto a sucrose step gradient containing equal volumes of 20%, 30%, 40%, and 55% w/v sucrose in PBS, and the gradient was centrifuged overnight at 100,000×g in a SW32Ti rotor at 4° C. The virus band was removed from the gradient and diluted in 5 volumes of PBS. Virus in the suspension was pelleted by centrifugation at 100,000×g in a

SW32Ti rotor for 2 hours at 4° C. The pellet supernatant was aspirated, and the virus pellet was incubated in 1 mL of PBS at 4° C. for 48 hours. The pellet was then resuspended by gentle pipetting and titered. HA assays showed that approximately 80% of the HA units present in the clarified cell supernatant were recovered as purified material following the procedure described above.

Virus Inactivation

Five hundred thousand HA units of virus was diluted to a final volume of 20 mL in PBS. A chemical reaction using formaldehyde was done to inactivate the viruses. Formaldehyde (2% v/v in PBS, Thermo Scientific) was added to a final concentration of 0.02%, and the solution was incubated at 22° C. for 13 hours while rotating at 90 rpm. Residual formaldehyde was neutralized by addition of sodium bisulfite at a 1:1 molar ratio of sulfite ion to formaldehyde. Virus inactivation was confirmed by sequential passage of the undiluted virus suspension on MDCK cells. HA assays performed on the virus suspension before and after inactivation showed that the inactivation process did not reduce the quantity of HA units when compared to that of the starting material.

Other inactivation methods involving chemical reactions, such as BPL (betapropiolactone) and BEI (binary ethylenimine) are used to inactivate the viruses.

These chemical inactivation methods produce enumerable structural changes, including for example, formation of new chemical bonds via chemical crosslinking, irreversible chemical alteration of the nucleic acid and protein coat (Uittenbogaard, 2011, Journal of Biological Chemistry, 286 (42): pp36198-36214; Gard, Bull. Wld Hlth Org., 1957, 17, 979-989).

Production of antibodies

Purified, inactivated H3N2 CIV #8 was mixed at a 1:1 ratio with TiterMax Gold adjuvant (Sigma-Aldrich). Ten female specific pathogen free New Zealand White rabbits 10-16 weeks of age (Harlan) were injected subcutaneously in the hind quarter with 3 or 4 doses of 1,500 HA units of virus per dose. One week after the last vaccine dose, animals were anesthetized, and blood was collected. Serum samples were tested for serum neutralizing antibody titers against H3N2 CIV.

Example 4 Viral Antigenic Analysis

Viral antigenic analysis

Antibodies that bind to the HA protein on the surface of influenza virus particles can inhibit particle-induced red blood cell (RBC) agglutination in the context of an HA assay if the antibodies are present in sufficient quantities. Thus, the hemagglutination inhibition (HAI) assay is routinely used to (i) measure the quantity or dilution of an antibody required to inhibit RBC agglutination by a given virus strain (i.e. HAI titer), and (ii) to evaluate the antigenic relatedness of HA proteins present in virus particles from two or more different virus strains. Tests of antigenic relatedness are performed with antisera raised against one virus strain (i.e. the homologous strain) and test the capacity of that antisera to inhibit RBC agglutination by heterologous virus strains. A heterologous virus is typically considered antigenically distinct from the homologous virus if the HAI titer for the heterologous virus is >8-fold less than the HAI titer for the homologous virus.

An HAI assay employing serum from dogs vaccinated with a canarypox vector expressing the HA protein from A/equine/Ohio/1/2003 H3N8 virus was used to test the antigenic relatedness of H3N8 and H3N2 CIV. This serum was previously determined to have an HAI titer of 512 when tested against A/canine/Colorado/8880/2006, a H3N8 canine influenza virus. The H3N2 CIV isolate #4 (egg passage 2) and A/canine/Colorado/8880/2006 were diluted to 8 HA units/25 μL in PBS and incubated with 2-fold dilutions of serum (25 μL per dilution) for 1 hour at 22° C. An equal volume (50 μL) of 0.5% chicken RBCs was added to each sample, and the solutions were incubated for an additional hour at 22° C. Antibodies in the serum failed to inhibit RBC agglutination by H3N2 CIV at all dilutions tested (1:8-1:2048, HAI titer <8). In contrast, the serum inhibited RBC agglutination by A/canine/Colorado/8880/2006 at a maximal dilution of 1:512 (HAI titer 512). Thus, antibodies in this serum that recognize the HA protein of a H3N8 CIV do not bind to the HA protein of H3N2 CIV with high affinity, suggesting that the viruses are antigenically distinct.

Example 5 Production of Vaccine Active Ingredient and Preparation of Vaccines

To produce CIV H3N2 active ingredient (AI) for the preparation of inactivated CIV H3N2 vaccine, MDCK cells are used to amplify the CIV H3N2 virus in roller bottles for multiple passages according to standard virus production procedures.

The vaccine is prepared by mixing the CIV H3N2 active ingredient with an adjuvant. The adjuvant is aluminum hydroxide (or aluminum phosphate) or LR4 emulsion (U.S. Pat. No. 7,691,368) which contains 12.5% of "incomplete LR emulsion" and 87.5% of aqueous phase (containing the active ingredient).

Example 6 Evaluation of H3N2 Monovalent Vaccine Safety and Immunogenicity in Animals

Vaccines were formulated as monovalent, aqueous solutions containing a 1× (1000 HAU) or 2× (1920 HAU) dose of whole, inactivated H3N2 CIV (PTA-122265 (virus ID #4)) and aluminum hydroxide (adjuvant). The placebo vaccine lacked antigen but contained adjuvant at the same concentration as the test vaccines.

Injection site reactions and clinical signs

Thirty CIV seronegative six-week old, commercial source beagles were randomized to three vaccination groups, containing 10 dogs each. Dogs in Groups 1 and 2 received 2× and 1× doses of the H3N2 CIV vaccine, respectively (Table 4). Dogs in Group 3 received a placebo vaccine. Each dog was vaccinated twice, 21 days apart, with 1 mL of vaccine, subcutaneously over the scapula. The dogs were monitored for injection site reactions and temperature elevations for three days after each vaccination and on days 14 and 27 (day prior to challenge). Blood samples were collected on days 0 (prior to vaccination), 7, 14, 21 (prior to vaccination), 27, and 35.

TABLE 4

Study Groups

Group	Vaccine	Vaccination Route	Vaccination Frequency	No. of dogs	Challenge
1	2X CIV	Subcutaneous	Twice, 21 days apart	10	H3N2 CIV
2	1X CIV	Subcutaneous	Twice, 21 days apart	10	H3N2 CIV

TABLE 4-continued

Study Groups					
Group	Vaccine	Vaccination Route	Vaccination Frequency	No. of dogs	Challenge
3	Placebo	Subcutaneous	Twice, 21 days apart	10	H3N2 CIV

Seven days after the second vaccination, the dogs were challenged with virulent H3N2 CIV (PTA-122266 (virus ID #8)) via aerosolization in a closed chamber using a commercial nebulizer. During challenge, dogs were randomized to challenge chambers using treatment group and litter as blocking factors such that each treatment group, litter, and sex (if possible) was represented in each challenge chamber run. After challenge, dogs were randomized to post-challenge (PC) pens such that each pen contained dogs from all three treatment groups and each chamber run from the challenge phase.

The dogs were observed for cough, fever, mucopurulent nasal discharge, and other clinical signs for seven days. Nasal swabs for virus isolation were collected on days 3, 4, and 5 PC. A dog was classified as having disease due to CIV if it developed cough in addition to either fever or mucopurulent nasal discharge. A dog was considered febrile when the rectal temperature was ≥39.7° C. and 0.5° C. above baseline (day 0 rectal temperature). The challenge was considered valid when at least 60% of the placebo vaccinated dogs developed disease due to CIV.

Following each vaccination of dogs with the H3N2 CIV vaccine at 1× and 2× doses, no systemic or injection site adverse reactions were observed. Prior to challenge, no dogs in any group showed clinical signs of disease. After challenge, the first clinical signs of disease (cough, mucopurulent nasal discharge, and fever) were observed two days post-challenge (DPC), similar to dogs with natural and experimental CIV infection (Table 5).

TABLE 5

Number of dogs expressing clinical signs of CIV disease post-challenge			
Vaccine Group	Cough	Fever	Mucopurulent Nasal Discharge
2X CIV (n = 10)	0	1	0
1X CIV (n = 10)	0	0	0
Placebo (n = 10)	9	5	10

Cough and mucopurulent nasal discharge were the most predominant clinical signs of disease, with most dogs in the placebo group coughing for 2 days and showing mucopurulent discharge for 4 days (Tables 6 and 7). Ninety percent (90%) of the dogs in the placebo group were observed with cough, and 100% of the placebo group developed mucopurulent nasal discharge. No animals in the 2×CIV or 1×CIV vaccine groups developed cough or mucopurulent discharge. The days with highest frequency were 3 DPC for cough (8 dogs) and 4 DPC for mucopurulent nasal discharge (10 dogs). Two days post-challenge was the day with the highest frequency of fever with 6 febrile dogs—five in the placebo group and one in the 2×CIV vaccine group. Most dogs had one instance of fever, while one dog in the placebo group had two days of fever (Table 8). Rectal temperatures for dogs with fever ranged from 39.7° C. to 40.4° C.

TABLE 6

Total days with cough				
	Number of days of cough			
Vaccine Group	2	3	4	5
2X CIV (n* = 0)	—	—	—	—
1X CIV (n = 0)	—	—	—	—
Placebo (n = 9)	6	1	1	1

n*: number of dogs (out of 10 dogs) with symptom.

TABLE 7

Total days with mucopurulent nasal discharge					
	Number of days of mucopurulent nasal discharge				
Vaccine Group	2	3	4	5	6
2X CIV (n* = 0)	—	—	—	—	—
1X CIV (n = 0)	—	—	—	—	—
Placebo (n = 10)	2	2	3	1	2

n*: number of dogs (out of 10 dogs) with symptoms.

TABLE 8

Total days with fever		
	Number of days of fever	
Vaccine Group	1	2
2X CIV (n* = 1)	1	—
1X CIV (n = 0)	—	—
Placebo (n = 5)	4	1

n*: number of dogs (out of 10 dogs) with symptoms.

Ninety percent (90%) of the dogs in the placebo group met the case definition for clinical disease, thereby validating the challenge. The incidence of disease in dogs administered the H3N2 CIV vaccines was significantly reduced compared to the placebo vaccinated dogs (p=0.0001) (Table 9). Clinical disease was prevented in 100% of the CIV vaccinates, underscoring the effectiveness of the vaccines at preventing clinical disease.

TABLE 9

2X dose and 1X dose H3N2 Vaccine Group Prevented Fractions for CIV disease			
Vaccine Group	Number of dogs with disease	P-value (Fisher's Exact Test)	Prevented Fraction (95% CI)*
2X CIV (n = 10)	0	0.0001	1.00
Placebo (n = 10)	9		(0.67, 1.00)
1X CIV (n = 10)	0	0.0001	1.00 (0.67, 1.00)

95% CI*: 95% confidence interval

Nasal shedding and serology

Fluids expressed from nasal swabs were inoculated into the allantoic cavity of 3 embryonic chicken eggs. Eggs were incubated for 3 days at 37° C. and tested for the presence of virus by HA assay. Specimens were considered positive for virus if at least 1 of 3 eggs contained detectable virus. Specimens were considered negative for virus if at least 2 eggs were viable at the time of testing, and no eggs contained detectable virus. Positive samples and samples for which 2 or 3 eggs were inviable at the time of testing were tested by $TCID_{50}$ assay.

Heat-inactivated serum samples were incubated with 2.5% v/v washed cRBCs for 30 min at room temperature, and cRBCs were pelleted by centrifugation. The pellet supernatant was used to perform the HAI assay using a final concentration of 0.25% v/v cRBCs. The HAI titer of each sample was recorded as the reciprocal of the highest serum dilution that completely inhibited cRBC agglutination by 8 HA units of H3N2 CIV. A titer of <4 was considered negative for CIV serum antibody.

Nasal swabs were collected to gauge the capability of the test vaccines to prevent viral shedding after challenge. In this study, the collection days also corresponded with the days of highest frequency of cough and mucopurulent nasal discharge. All dogs in the placebo group exhibited nasal shedding of CIV, and shedding was significantly reduced in the 2× and 1× dose groups on each collection day (Table 10). The reduction in shedding in the test vaccine groups equated to an 80% preventable fraction (Table 11). Similarly, the CIV vaccines administered in this study reduced shedding to undetectable levels in 100% of the CIV vaccinated dogs at 5 DPC.

TABLE 10

Incidence of nasal CIV shedding

Vaccine Group	Number of dogs with positive nasal shedding 3 DPC (Day 31)	4 DPC (Day 32)	5 DPC (Day 33)
2X CIV (n = 10)	$0^{§a}$	$2^{§b}$	$0^{§a}$
1X CIV (n = 10)	$1^{§c}$	$1^{§c}$	$0^{§a}$
Placebo (n = 10)	10	10	10

§significant difference between test vaccine and placebo
$^{a}p < 0.0001$
$^{b}p = 0.0007$
$^{c}p = 0.0001$

TABLE 11

2X dose and 1X dose H3N2 Vaccine Group Prevented Fractions for nasal shedding

Vaccine Group	Number of dogs with positive nasal shedding	P-value (Fisher's Exact Test)	Prevented Fraction (95% CI)
2X CIV (n = 10)	2	0.0007	0.80 (0.46, 0.96)
Placebo (n = 10)	10		
1X CIV (n = 10)	2	0.0007	0.80 (0.46, 0.96)

This study is consistent with the observation that dogs not showing clinical signs of disease may still shed virus. The study found that 90% of the placebo dogs exhibited clinical disease, while 100% of the placebo dogs shed infectious virus. This result emphasizes the importance of limiting virus shedding as part of strategies to control virus transmission.

All animals were seronegative prior to the initiation of the study, and all dogs in the placebo group were seronegative prior to challenge (Table 12). On day 21, the proportion of dogs that seroconverted in the 2×CIV group was significantly higher than in the placebo group (p=0.0031), and on day 27, all dogs in the 2× and 1×CIV groups had seroconverted. Hence, seroconversion correlated with protection of the vaccinated dogs from clinical disease.

TABLE 12

Dogs seropositive (≥4) for CIV antibodies

Vaccine Group	Number of dogs seroconverted Day 0*	Day 7	Day 14	Day 21*	Day 27#	Day 35†
2X CIV (n = 10)	0	0	1	$7^{§a}$	$10^{§b}$	10
1X CIV (n = 10)	0	0	0	2	$10^{§b}$	10
Placebo (n = 10)	0	0	0	0	0	6

*Vaccination days-sample collected prior to vaccination
#One day prior to challenge
†Seven days post-challenge
§significant difference between test vaccine and placebo
$^{a}p = 0.0031$
$^{b}p < 0.0001$

This study demonstrates that vaccination of dogs with an inactivated H3N2 CIV vaccine was well tolerated, produced seroconversion, and successfully protected 100% of vaccinates from clinical disease caused by challenge with virulent virus. Notably, the vaccines also reduced nasal shedding of infectious challenge virus, substantiating their use as safe and effective measures to alleviate signs of disease caused by CIV and potentially control virus transmission.

Example 6 Virus Propagation and Genomic RNA of H3N8 CIV Strains

Viruses from H3N8 CIV strains CT/85863/11, NY/120106/11 and WY/86033/07 were isolated in specific pathogen free embryonic chicken eggs and subsequently propagated in eggs or MDCK cells at 37° C. for 72 hours (eggs) or 36-48 hours (cells). Viruses were titrated by HA or $TCID_{50}$ assay as described for H3N2 CIV. The nucleotide sequences of the viral HA and NA genes from each virus strain were determined as for H3N2 CIV using the gene specific primers in FIG. **1**.

The HA and NA polynucleotide and protein sequences for H3N8 CIV strains CT/85863/11, NY/120106/11 and WY/86033/07 are designated SEQ ID NOs:30-35 and 37-42 as shown in FIGS. **1** and **5**.

Example 7 Preparation of Bivalent H3N2 and H3N8 CIV Vaccines

Viruses used in vaccine preparation were produced by infection of confluent monolayers of MDCK cells in roller bottle cultures at MOIs ranging from 0.0001 to 0.00001 in 200 mL of MEM supplemented with 1-2 ug/mL of porcine-origin trypsin (Sigma-Aldrich) per roller bottle. Cultures were incubated at 37° C. and 0.5 rpm and harvested 24 hours (H3N2 CIV) or 36-42 hours (H3N8 CIV) after inoculation of the cells with virus. Cell debris was removed from the virus suspension by centrifugation at 1,000×g or filtration through membranes containing 5 μM diameter pores. To inactivate infectious virus in the suspensions, formaldehyde was added to a final (v/v) concentration of 0.04%, and the reactions were incubated for 24 hours at 37° C. Inactivated virus preparations were stored at 4° C. until further use. In some cases, inactivated viral antigens were concentrated by ultrafiltration using polysulfone membranes containing 10 kDa pores. Vaccine antigens were confirmed to be fully inactivated and sterile prior to vaccine formulation.

Bivalent vaccines were prepared by mixing the desired quantity of each inactivated vaccine antigen with an adjuvant overnight at 4° C. The adjuvant is aluminum hydroxide (or aluminum phosphate) or LR4 emulsion (U.S. Pat. No. 7,691,368) which contains 12.5% of "incomplete LR emulsion" and 87.5% of aqueous phase (containing the active ingredient). Prepared vaccines were aliquoted into glass vials, sealed, and stored at 4° C. until use.

Example 8 Evaluation of H3N2/H3N8 Bivalent Vaccine Safety and Immunogenicity in Animals

Example 8.1 Efficacy of a Canine Influenza Virus H3N2/H3N8 Combination Vaccine Against H3N8 Challenge in Dogs

The goal of the study is to evaluate the serological response, adverse events (interference) and efficacy of an injectable canine influenza virus H3N2/H3N8 combination vaccine in a vaccination-challenge model in dogs.

Thirty CIV seronegative six-week (+/−6 days) old, commercial source beagles were randomized to four vaccination groups as shown in Table 14 in one study. Dogs in Groups A and B received 1× and 2× doses of the H3N2/H3N8 combo CIV vaccine, respectively (Tables 13 and 14). Dogs in group C received a dose of H3N2 CIV vaccine. Dogs in Group D received a placebo vaccine. In another study, thirty CIV seronegative six-week (+/−6 days) old, commercial source beagles were randomized to two vaccination groups as shown in Table 15. Dogs in Group A received one dose of the H3N2/H3N8 combo CIV vaccine. Dogs in Group B received a placebo vaccine. Each dog was vaccinated twice, 21 days apart, with 1 mL of vaccine, subcutaneously. The dogs were monitored for injection site reactions and temperature elevations for three days after each vaccination and on days 7, 14, 21 (prior to vaccination), 22-24, 28 (prior to challenge) and 38 (end of study). Blood samples were collected on days 0 (prior to vaccination), 7, 14, 21 (prior to vaccination), 28 (prior to challenge), and 38 (end of study).

TABLE 13

Vaccine formulation

	H3N2/H3N8 Low dose(1X H3N2 and 1X H3N8)	H3N2/H3N8 High dose (1X H3N2 and 2X H3N8)	CIV H3N2	Control
Vaccine formulation	Inactivated H3N2* and H3N8** adjuvanted with $Al(OH)_3$	Inactivated H3N2 and H3N8 adjuvanted with $Al(OH)_3$	Inactivated H3N2 adjuvanted with $Al(OH)_3$	PBS adjuvanted with $Al(OH)_3$
dose	1000 HAU of H3N2 and 750 HAU of H3N8 per dose; 1 ml = dose	1000 HAU of H3N2 and 1500 HAU of H3N8 per dose; 1 ml = dose	1000 HAU of H3N2 per dose; 1 ml = dose	1 ml = 1 dose

H3N2*: H3N2 CIV strain PTA-122265 (virus ID #4)
H3N8**: H3N8 CIV strain A/Ca/CT/85863/11

TABLE 14

Study design

Group	Vaccine	Dose Volume	Route of Adminis-tration	Frequency of Administration	Number of animals
A	Low dose H3N2/H3N8	1 ml	SC	Twice, 21 days apart	10
B	High dose H3N2/H3N8	1 ml	SC	Twice, 21 days apart	5
C	CIV H3N2	1 ml	SC	Twice, 21 days apart	10
D	Control	1 ml	SC	Twice, 21 days apart	5

TABLE 15

Study design

Group	Vaccine	Dose Volume	Route of Adminis-tration	Frequency of Administration	Number of animals
A	H3N2/H3N8*	1 ml	SC	Twice, 21 days apart	15
B	Control**	1 ml	SC	Twice, 21 days apart	15

H3N2/H3N8*: Inactivated H3N2 and H3N8 adjuvanted with $Al(OH)_3$; 1000 HAU of H3N2 and 750 HAU of H3N8 per dose; 1 ml = 1 dose
Control**: PBS adjuvanted with $Al(OH)_3$; 1 ml = 1 dose

Seven days after the second vaccination (day 28), the dogs were challenged with virulent H3N8 CIV strains WY/86033/07 and NY/120106/11 via aerosolization in a closed chamber using a commercial nebulizer. During challenge, dogs were randomized to challenge chambers using treatment group and litter as blocking factors such that each treatment group, litter, and sex (if possible) was represented in each challenge chamber run. After challenge, dogs were randomized to post-challenge (PC) pens such that each pen contained dogs from all three treatment groups and each chamber run from the challenge phase.

The dogs were observed for cough, fever, mucopurulent nasal discharge, and other clinical signs for ten days. Nasal swabs for virus isolation were collected three to six times after PC. A dog was classified as having disease due to CIV if it developed cough in addition to either fever or mucopurulent nasal discharge. A dog was considered febrile when the rectal temperature was ≥39.7° C. and 0.5° C. above baseline (day 0 rectal temperature). The challenge was considered valid when at least 60% of the placebo vaccinated dogs developed disease due to CIV.

The results show that vaccination of dogs with an inactivated H3N2/H3N8 combination vaccine is well tolerated, produced seroconversion, and successfully protected vaccinates from clinical disease caused by challenge with virulent H3N8 virus. The H3N2/H3N8 combination vaccine also demonstrates the lack of interference between the inactivated H3N2 vaccine and the inactivated H3N8 vaccine when formulated together in a combination vaccine.

Example 8.2 Efficacy of a Canine Influenza Virus H3N2/H3N8 Combination Vaccine Against H3N2 Challenge in Dogs

The goal of the study is to evaluate the efficacy of an injectable canine influenza virus H3N2/H3N8 combination vaccine against lung lesions induced by CIV H3N2 in a vaccination-challenge model.

Thirty CIV seronegative six-week (+/−6 days) old, commercial source beagles were randomized to two vaccination groups as shown in Table 16. Dogs in Group A received one dose of the H3N2/H3N8 combo CIV vaccine. Dogs in Group B received a placebo vaccine. Each dog was vaccinated twice, 21 days apart, with 1 mL of vaccine, subcutaneously. The dogs were monitored for injection site reactions and temperature elevations for three days after each vaccination and on days 7, 14, 21 (prior to vaccination), 22-24, 28 (prior to challenge), 29-35, and 36 (end of study). Blood samples were collected on days 0 (prior to vaccination), 7, 14, 21 (prior to vaccination), 28 (prior to challenge), and 38 (end of study).

TABLE 16

Study design

Group	Vaccine	Dose Volume	Route of Adminis-tration	Frequency of Administration	Number of animals
A	H3N2/H3N8* Low dose	1 ml	SC	Twice, 21 days apart	15
B	Control**	1 ml	SC	Twice, 21 days apart	15

H3N2/H3N8*: Inactivated H3N2 and H3N8 adjuvanted with $Al(OH)_3$; 1000 HAU of H3N2 and 750 HAU of H3N8 per dose; 1 ml = 1 dose
Control**: PBS adjuvanted with $Al(OH)_3$; 1 ml = 1 dose

One week after the second vaccination the dogs are challenged with CIV H3N2 (PTA-122266 (virus ID #8) via aerosolization as described in Example 8.1. The dogs are observed for cough, fever, mucopurulent nasal discharge, and other clinical signs for seven days. Nasal swabs for virus isolation are collected three to six times after PC. Data are analyzed based on the criteria set out in Example 8.1.

The results show that vaccination of dogs with an inactivated H3N2/H3N8 combination vaccine is well tolerated, produced seroconversion, and successfully protected vaccinates from clinical disease caused by challenge with virulent H3N2 virus. The H3N2/H3N8 combination vaccine also demonstrates the lack of interference between the inactivated H3N2 vaccine and the inactivated H3N8 vaccine when formulated together in a combination vaccine.

Having thus described in detail embodiments of the present disclosure, it is to be understood that the disclosure defined by the above examples is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present disclosure.

All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

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aggattcaag accttgagag atacgttgaa gacacaaaag tagatctttg gtcttacaat 1320

gccgagcttc ttgttgcctt agaaaaccag aacacaattg atttaactga ttcagaaatg 1380

aacaaattgt ttgaaaagac taggaggcaa ttgagggaaa atgctgaaga catgggcaat 1440

ggctgcttca agatatacca caagtgtgac aatgcttgca tagaatcgat tagaaacgga 1500

acttatgacc ataacatata tagagatgag gcagtgaaca atcggttcca gatcaaaggt 1560

gttgagctaa agtctggata caaagactgg atcttatgga tttcctttgc catatcatgc 1620

tttttgcttt gtgttgtctt gctgggtttc attatgtggg cctgccagag aggcaacatt 1680

aggtgcaaca tttgcatt 1698

<210> SEQ ID NO 4
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: HA protein from #4 (in MDCK cells)

<400> SEQUENCE: 4

```
Met Lys Thr Val Ile Ala Leu Ser Tyr Ile Phe Cys Leu Ala Phe Gly
1               5                   10                  15

Gln Asn Leu Leu Gly Asn Glu Asn Asn Ala Ala Thr Leu Cys Leu Gly
            20                  25                  30

His His Ala Val Pro Asn Gly Thr Met Val Lys Thr Ile Thr Asp Asp
        35                  40                  45

Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Asn Ser Ser Thr
    50                  55                  60

Gly Lys Ile Cys Asn Asn Pro His Lys Ile Leu Asp Gly Arg Asp Cys
65                  70                  75                  80

Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro His Cys Asp Val Phe Gln
                85                  90                  95

Asn Glu Thr Trp Asp Leu Phe Val Glu Arg Ser Asn Ala Phe Ser Asn
            100                 105                 110

Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val
        115                 120                 125

Ala Ser Ser Gly Thr Leu Glu Phe Ile Thr Glu Gly Phe Thr Trp Ala
    130                 135                 140

Gly Val Thr Gln Asn Gly Gly Ser Gly Ala Cys Lys Arg Gly Pro Ala
145                 150                 155                 160

Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Asn Thr
                165                 170                 175

Tyr Pro Val Leu Asn Val Thr Met Pro Asn Asn Asn Asn Phe Asp Lys
            180                 185                 190

Leu Tyr Ile Trp Gly Val His His Pro Ser Thr Asn Gln Glu Gln Thr
        195                 200                 205

Ser Leu Tyr Ile Gln Ala Ser Gly Arg Val Lys Val Ser Thr Arg Arg
    210                 215                 220

Ser Gln Gln Thr Ile Ile Pro Asn Ile Gly Ser Arg Pro Leu Val Arg
225                 230                 235                 240

Gly Gln Ser Gly Arg Ile Ser Val Tyr Trp Thr Ile Val Lys Pro Gly
                245                 250                 255

Asp Ile Leu Val Ile Asn Ser Asn Gly Asn Leu Ile Ala Pro Arg Gly
            260                 265                 270

Tyr Phe Lys Met His Ile Gly Lys Ser Ser Ile Met Arg Ser Asp Ala
        275                 280                 285

Pro Ile Asp Thr Cys Ile Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile
    290                 295                 300

Pro Asn Glu Lys Pro Phe Gln Asn Val Asn Lys Ile Thr Tyr Gly Ala
305                 310                 315                 320

Cys Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met
                325                 330                 335

Arg Asn Val Pro Glu Arg Gln Thr Arg Gly Leu Phe Gly Ala Ile Ala
            340                 345                 350

Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly
        355                 360                 365

Phe Arg His Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys
    370                 375                 380

Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val
385                 390                 395                 400

Ile Glu Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser
                405                 410                 415
```

```
Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Arg Tyr Val Glu Asp Thr
            420                 425                 430

Lys Val Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu
        435                 440                 445

Asn Gln Asn Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe
    450                 455                 460

Glu Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn
465                 470                 475                 480

Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Glu Ser
                485                 490                 495

Ile Arg Asn Gly Thr Tyr Asp His Asn Ile Tyr Arg Asp Glu Ala Val
            500                 505                 510

Asn Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys
        515                 520                 525

Asp Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys
    530                 535                 540

Val Val Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Arg Gly Asn Ile
545                 550                 555                 560

Arg Cys Asn Ile Cys Ile
                565
```

<210> SEQ ID NO 5
<211> LENGTH: 1698
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding HA protein from #8 (in eggs)

<400> SEQUENCE: 5

```
atgaaaactg ttattgcttt aagctatatt ttctgcctgg cttttggtca gaatcttcta     60

ggaaatgaaa ataatgctgc aacactatgc ctgggacatc atgcagtgcc gaacgggaca    120

atggtgaaaa ctatcacaga cgatcaaatt gaggtgacca acgccaccga gctagtccaa    180

aaccccctcaa cagggaaaat atgcaacaat ccccacaaga ttcttgatgg gagggactgc    240

acactaatag atgccctact aggggaccca cactgtgacg tcttccaaaa tgagacatgg    300

gacctttttg tggaacgaag caatgctttt agcaattgtt acccttatga tgtaccagac    360

tatgcatccc tccgatccat agttgcatca tcaggcacat tggagttcat cactgaaggt    420

ttcacttggg caggagtaac tcaaaatgga ggaagcggtg cttgtaaaag gggacctgct    480

aatagtttct tcagtagatt aaattggtta actaaatcag gaaatacata tccagtgttg    540

aatgtgacta tgccaaacaa caacaatttc gacaaattat acatttgggg agttcatcac    600

ccaagcacta atcaagaaca aaccagcctg tatattcagg cctcaggaag agtcacagtc    660

tctaccagga gaagccaaca gaccataatc ccaaacattg gatctagacc cttggtaagg    720

ggccaatctg gcagaataag cgtatattgg acaatagtca aacctggaga catactggta    780

ataaacagta atggaaacct aatcgctcct cgaggatact tcaaaatgca cattgggaaa    840

agctcaataa tgagatcaga tgcacctatt gacacctgca tttccgaatg tatcaccccg    900

aacgggagca tccccaatga aaagcccttc caaaatgtaa acaagatcac atacggagca    960

tgtcccaaat atgttaagca aaacaccttg aaactggcaa caggaatgcg gaatgtccct   1020

gagaggcaaa ccagaggcct gttcggcgca atagcaggct tcatagaaaa tggatgggaa   1080

gggatggtag acggttggta tggcttcagg caccaaaatt ccgaaggtac aggacaagca   1140

gcagacctta aaagcactca ggcagccatt gaccagatta atgggaaatt gaacagagtg   1200
```

```
attgaaaaaa cgaatgagaa gttccatcaa attgaaaagg agttttccga agtagaaggg   1260

aggattcaag accttgagag atacgttgaa gacacaaaag tagatctttg gtcttacaat   1320

gccgagcttc ttgttgcctt agaaaaccag aacacaattg atttaactga ttcagaaatg   1380

aacaaattgt ttgaaaagac taggaggcaa ttgagggaaa atgctgaaga catgggcaat   1440

ggctgcttca agatatacca caagtgtgac aatgcttgca tagaatcgat tagaaacgga   1500

acttatgacc ataacatata tagagatgag gcagtgaaca atcggttcca gatcaaaggt   1560

gttgagctaa agtctggata caaagactgg atcttgtgga tttcctttgc catatcatgc   1620

tttttgcttt gtgttgtctt gctgggtttc attatgtggg cctgccagag aggcaacatt   1680

aggtgcaaca tttgcatt                                                 1698


<210> SEQ ID NO 6
<211> LENGTH: 566
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein from #8 (in eggs)

<400> SEQUENCE: 6

Met Lys Thr Val Ile Ala Leu Ser Tyr Ile Phe Cys Leu Ala Phe Gly
1               5                   10                  15

Gln Asn Leu Leu Gly Asn Glu Asn Asn Ala Ala Thr Leu Cys Leu Gly
            20                  25                  30

His His Ala Val Pro Asn Gly Thr Met Val Lys Thr Ile Thr Asp Asp
        35                  40                  45

Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Asn Pro Ser Thr
    50                  55                  60

Gly Lys Ile Cys Asn Asn Pro His Lys Ile Leu Asp Gly Arg Asp Cys
65                  70                  75                  80

Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro His Cys Asp Val Phe Gln
                85                  90                  95

Asn Glu Thr Trp Asp Leu Phe Val Glu Arg Ser Asn Ala Phe Ser Asn
            100                 105                 110

Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val
        115                 120                 125

Ala Ser Ser Gly Thr Leu Glu Phe Ile Thr Glu Gly Phe Thr Trp Ala
    130                 135                 140

Gly Val Thr Gln Asn Gly Gly Ser Gly Ala Cys Lys Arg Gly Pro Ala
145                 150                 155                 160

Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Asn Thr
                165                 170                 175

Tyr Pro Val Leu Asn Val Thr Met Pro Asn Asn Asn Asn Phe Asp Lys
            180                 185                 190

Leu Tyr Ile Trp Gly Val His His Pro Ser Thr Asn Gln Glu Gln Thr
        195                 200                 205

Ser Leu Tyr Ile Gln Ala Ser Gly Arg Val Thr Val Ser Thr Arg Arg
    210                 215                 220

Ser Gln Gln Thr Ile Ile Pro Asn Ile Gly Ser Arg Pro Leu Val Arg
225                 230                 235                 240

Gly Gln Ser Gly Arg Ile Ser Val Tyr Trp Thr Ile Val Lys Pro Gly
                245                 250                 255

Asp Ile Leu Val Ile Asn Ser Asn Gly Asn Leu Ile Ala Pro Arg Gly
            260                 265                 270
```

```
Tyr Phe Lys Met His Ile Gly Lys Ser Ser Ile Met Arg Ser Asp Ala
        275                 280                 285

Pro Ile Asp Thr Cys Ile Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile
    290                 295                 300

Pro Asn Glu Lys Pro Phe Gln Asn Val Asn Lys Ile Thr Tyr Gly Ala
305                 310                 315                 320

Cys Pro Lys Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met
                325                 330                 335

Arg Asn Val Pro Glu Arg Gln Thr Arg Gly Leu Phe Gly Ala Ile Ala
            340                 345                 350

Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly
        355                 360                 365

Phe Arg His Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys
    370                 375                 380

Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val
385                 390                 395                 400

Ile Glu Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser
                405                 410                 415

Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Arg Tyr Val Glu Asp Thr
            420                 425                 430

Lys Val Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu
        435                 440                 445

Asn Gln Asn Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe
    450                 455                 460

Glu Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn
465                 470                 475                 480

Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Glu Ser
                485                 490                 495

Ile Arg Asn Gly Thr Tyr Asp His Asn Ile Tyr Arg Asp Glu Ala Val
            500                 505                 510

Asn Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys
        515                 520                 525

Asp Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys
    530                 535                 540

Val Val Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Arg Gly Asn Ile
545                 550                 555                 560

Arg Cys Asn Ile Cys Ile
                565
```

<210> SEQ ID NO 7
<211> LENGTH: 1407
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein from #4 (in eggs)

<400> SEQUENCE: 7

```
atgaacccaa atcaaaagat aatagcaata gggtctgtct ctctaaccat tgcaacagta      60

tgtttcctct tacagattgc catcctagca acaactgtga cactgtactt caagcaaaat     120

gaatgcaaca tcccctcgaa cagtcaagta gtgccatgta aaccaatcat aatagaaagg     180

aacataacag aggtagtata tttgaataat actaccatag aaaaagaaat ttgctccgta     240

gtgctagaat acaggaactg gtcgaaaccg cagtgtcaaa ttacaggatt tgctcctttc     300

tccaaggaca actcaatccg actctccgct ggtggggaca tttgggtaac aagggaacct     360
```

-continued

tatgtgtcat gcgaccacag caaatgttat cagtttgcac ttgggcaggg gaccacgctg 420

aacaataaac actcaaacag cacaatacat gataggacct ctcatcgaac tcttttaatg 480

aatgagttgg gtgttccgtt tcatttggga accaaacaag tgtgcatagc atggtccagt 540

tcaagttgtc acgatgggaa agcatggtta catgtttgtg tcactggaga tgatagaaat 600

gcgactgcta gtttcgttta taatggaatg cttgttgaca gtattggttc atggtctcga 660

aatatcctca gaactcaaga gtcagaatgt gtttgcatca atgggacttg tacagtagta 720

atgactgatg gaagtgcatc aggaagggct gatactagaa tactattcat cagagagggg 780

aaaattatcc atattagccc attgtcaggg agtgctcaac acatagagga atgttcctgt 840

tatccccgat atccaaatgt tagatgtgtt tgcagagaca attggaaggg ctccaatagg 900

cccgttatag atataaatat ggcagattat aacatcaatt ccagttatgt ctgttcagga 960

cttgttggcg atacaccaag gaatgatgat agctctagca gcagtaactg caaggatcct 1020

aataatgaga gagggaatcc aggagtgaag ggtgggcct ttgataatga taatgacgtt 1080

tggatgggga ggacaatcag caaagattta cgttcaggtt atgagacttt caaggtcatt 1140

ggtggctgga ccactgctaa ttccaagtca caggtcaata gacaagtcat agttgacaat 1200

aataactggt ctggttattc tggtattttc tccgttgaag gcaaaagctg tgttaatagg 1260

tgtttttatg tggagttgat aagaggaggg ccacaagaga ctagagtatg gtggacttca 1320

aatagcattg tcgtattttg tggtacttct ggtacctatg gaacaggctc atggcctgat 1380

ggggcgaata ttaacttcat gcctata 1407

<210> SEQ ID NO 8
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein from #4 (in eggs)

<400> SEQUENCE: 8

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Ser Val Ser Leu Thr
1 5 10 15

Ile Ala Thr Val Cys Phe Leu Leu Gln Ile Ala Ile Leu Ala Thr Thr
20 25 30

Val Thr Leu Tyr Phe Lys Gln Asn Glu Cys Asn Ile Pro Ser Asn Ser
35 40 45

Gln Val Val Pro Cys Lys Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
50 55 60

Val Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Ser Val
65 70 75 80

Val Leu Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
85 90 95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
100 105 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp His Ser Lys
115 120 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Asn Asn Lys His
130 135 140

Ser Asn Ser Thr Ile His Asp Arg Thr Ser His Arg Thr Leu Leu Met
145 150 155 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Ile
165 170 175

```
Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
            180                 185                 190

Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Val Tyr Asn
        195                 200                 205

Gly Met Leu Val Asp Ser Ile Gly Ser Trp Ser Arg Asn Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
                245                 250                 255

Ile Arg Glu Gly Lys Ile Ile His Ile Ser Pro Leu Ser Gly Ser Ala
            260                 265                 270

Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asn Val Arg
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp
    290                 295                 300

Ile Asn Met Ala Asp Tyr Asn Ile Asn Ser Ser Tyr Val Cys Ser Gly
305                 310                 315                 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Ser Ser Asn
                325                 330                 335

Cys Lys Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp
            340                 345                 350

Ala Phe Asp Asn Asp Asn Asp Val Trp Met Gly Arg Thr Ile Ser Lys
        355                 360                 365

Asp Leu Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Thr
    370                 375                 380

Thr Ala Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn
385                 390                 395                 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser
                405                 410                 415

Cys Val Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Gly Pro Gln
            420                 425                 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
        435                 440                 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
    450                 455                 460

Asn Phe Met Pro Ile
465
```

<210> SEQ ID NO 9
<211> LENGTH: 1407
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein from #4 (in MDCK cells)

<400> SEQUENCE: 9

```
atgaacccaa atcaaaagat aatagcaata gggtctgtct ctctaaccat tgcaacagta      60

tgtttcctct tacagattgc catcctagca acaactgtga cactgtactt caagcaaaat     120

gaatgcaaca tcccctcgaa cagtcaagta gtgccatgta aaccaatcat aatagaaagg     180

aacataacag aggtagtata tttgaataat actaccatag aaaaagaaat ttgctccgta     240

gtgctagaat acaggaactg gtcgaaaccg cagtgtcaaa ttacaggatt tgctcctttc     300

tccaaggaca actcaatccg actctccgct ggtggggaca tttgggtaac aagggaacct     360
```

```
tatgtgtcat gcgaccacag caaatgttat cagtttgcac ttgggcaggg gaccacgctg    420

aacaataaac actcaaacag cacaatacat gataggacct ctcatcgaac tcttttaatg    480

aatgagttgg gtgttccgtt tcatttggga accaaacaag tgtgcatagc atggtccagt    540

tcaagttgtc acgatgggaa agcatggtta catgtttgtg tcactggaga tgatagaaat    600

gcgactgcta gtttcgttta taatggaatg cttgttgaca gtattggttc atggtctcga    660

aatatcctca gaactcaaga gtcagaatgt gtttgcatca atgggacttg tacagtagta    720

atgactgatg gaagtgcatc aggaagggct gatactagaa tactattcat cagagagggg    780

aaaattatcc atattagccc attgtcaggg agtgctcaac acatagagga atgttcctgt    840

tatccccgat atccaaatgt tagatgtgtt tgcagagaca attggaaggg ctccaatagg    900

cccgttatag atataaatat ggcagattat aacatcaatt ccagttatgt ctgttcagga    960

cttgttggcg atacaccaag gaatgatgat agctctagca gcagtaactg caaggatcct   1020

aataatgaga gagggaatcc aggagtgaag gggtgggcct ttgataatga taatgacgtt   1080

tggatgggga ggacaatcag caaagattta cgttcaggtt atgagacttt caaggtcatt   1140

ggtggctgga ccactgctaa ttccaagtca caggtcaata gacaagtcat agttgacaat   1200

aataactggt ctggttattc tggtattttc tccgttgaag gcaaaagctg tgttaatagg   1260

tgtttttatg tggagttgat aagaggaggg ccacaagaga ctagagtatg gtggacttca   1320

aatagcattg tcgtattttg tggtacttct ggtacctatg gaacaggctc atggcctgat   1380

ggggcgaata ttaacttcat gcctata                                       1407
```

```
<210> SEQ ID NO 10
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein from #4 (in MDCK cells)

<400> SEQUENCE: 10

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Ser Val Ser Leu Thr
1               5                   10                  15

Ile Ala Thr Val Cys Phe Leu Leu Gln Ile Ala Ile Leu Ala Thr Thr
            20                  25                  30

Val Thr Leu Tyr Phe Lys Gln Asn Glu Cys Asn Ile Pro Ser Asn Ser
        35                  40                  45

Gln Val Val Pro Cys Lys Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
    50                  55                  60

Val Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Ser Val
65                  70                  75                  80

Val Leu Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
                85                  90                  95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
            100                 105                 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp His Ser Lys
        115                 120                 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Asn Asn Lys His
    130                 135                 140

Ser Asn Ser Thr Ile His Asp Arg Thr Ser His Arg Thr Leu Leu Met
145                 150                 155                 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Ile
                165                 170                 175
```

```
Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
            180                 185                 190

Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Val Tyr Asn
        195                 200                 205

Gly Met Leu Val Asp Ser Ile Gly Ser Trp Ser Arg Asn Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
                245                 250                 255

Ile Arg Glu Gly Lys Ile Ile His Ile Ser Pro Leu Ser Gly Ser Ala
            260                 265                 270

Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asn Val Arg
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp
    290                 295                 300

Ile Asn Met Ala Asp Tyr Asn Ile Asn Ser Ser Tyr Val Cys Ser Gly
305                 310                 315                 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Ser Ser Asn
                325                 330                 335

Cys Lys Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp
            340                 345                 350

Ala Phe Asp Asn Asp Asn Asp Val Trp Met Gly Arg Thr Ile Ser Lys
        355                 360                 365

Asp Leu Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Thr
    370                 375                 380

Thr Ala Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn
385                 390                 395                 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser
                405                 410                 415

Cys Val Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Gly Pro Gln
            420                 425                 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
        435                 440                 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
    450                 455                 460

Asn Phe Met Pro Ile
465
```

<210> SEQ ID NO 11
<211> LENGTH: 1407
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein from #8 (in eggs)

<400> SEQUENCE: 11

```
atgaacccaa atcaaaagat aatagcaata gggtctgtct ctctaaccat tgcaacagta      60

tgtttcctct tacagattgc catcctagca acaactgtga cactgtactt caagcaaaat     120

gaatgcaaca tcccctcgaa cagtcaagta gtgccatgta aaccaatcat aatagaaagg     180

aacataacag aggtagtata tttgaataat actaccatag aaaaagaaat ttgctccgta     240

gtgctagaat acaggaactg gtcgaaaccg cagtgtcaaa ttacaggatt tgctcctttc     300

tccaaggaca actcaatccg actctccgct ggtggggaca tttgggtaac aagggaacct     360
```

```
tatgtgtcat gcgaccacag caaatgttat cagtttgcac ttgggcaggg gaccacgctg    420

aacaataaac actcaaacag cacaatacat gataggacct ctcatcgaac tcttttaatg    480

aatgagttgg gtgttccgtt tcatttggga accaaacaag tgtgcatagc atggtccagt    540

tcaagttgtc acgatgggaa agcatggtta catgtttgtg tcactggaga tgatagaaat    600

gcgactgcta gtttcgttta taatggaatg cttgttgaca gtattggttc atggtctcga    660

aatatcctca gaactcaaga gtcagaatgt gtttgcatca atgggacttg tacagtagta    720

atgactgatg gaagtgcatc aggaagggct gatactagaa tactattcat cagagagggg    780

aaaattatcc atattagccc attgtcaggg agtgctcaac acatagagga atgttcctgt    840

tatccccgat atccaaatgt tagatgtgtt tgcagagaca attggaaggg ctccaatagg    900

cccgttatag atataaatat ggcagattat aacatcaatt ccagttatgt ctgttcagga    960

cttgttggcg atacaccaag gaatgatgat agctctagca gcagtaactg caaggatcct   1020

aataatgaga gagggaatcc aggagtgaag ggtgggcct ttgataatga taatgacgtt   1080

tggatgggga ggacaatcag caaagattta cgttcaggtt atgagacttt caaggtcatt   1140

ggtggctgga ccactgctaa ttccaagtca caggtcaata gacaagtcat agttgacaat   1200

aataactggt ctggttattc tggtattttc tccgttgaag gcaaaagctg tgttaatagg   1260

tgtttttatg tggagttgat aaggggaggg ccacaagaga ctagagtatg gtggacttca   1320

aatagcattg tcgtattttg tggtacttct ggtacctatg gaacaggctc atggcctgat   1380

ggagcgaata ttaacttcat gcctata                                       1407
```

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<210> SEQ ID NO 12
<211> LENGTH: 469
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein from #8 (in eggs)

<400> SEQUENCE: 12

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Ser Val Ser Leu Thr
1               5                   10                  15

Ile Ala Thr Val Cys Phe Leu Leu Gln Ile Ala Ile Leu Ala Thr Thr
            20                  25                  30

Val Thr Leu Tyr Phe Lys Gln Asn Glu Cys Asn Ile Pro Ser Asn Ser
        35                  40                  45

Gln Val Val Pro Cys Lys Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
    50                  55                  60

Val Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Ser Val
65                  70                  75                  80

Val Leu Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
                85                  90                  95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
            100                 105                 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp His Ser Lys
        115                 120                 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Asn Asn Lys His
    130                 135                 140

Ser Asn Ser Thr Ile His Asp Arg Thr Ser His Arg Thr Leu Leu Met
145                 150                 155                 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Ile
                165                 170                 175
```

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Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
            180                 185                 190

Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Val Tyr Asn
        195                 200                 205

Gly Met Leu Val Asp Ser Ile Gly Ser Trp Ser Arg Asn Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe
                245                 250                 255

Ile Arg Glu Gly Lys Ile Ile His Ile Ser Pro Leu Ser Gly Ser Ala
            260                 265                 270

Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asn Val Arg
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp
    290                 295                 300

Ile Asn Met Ala Asp Tyr Asn Ile Asn Ser Ser Tyr Val Cys Ser Gly
305                 310                 315                 320

Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Ser Ser Asn
                325                 330                 335

Cys Lys Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp
            340                 345                 350

Ala Phe Asp Asn Asp Asn Asp Val Trp Met Gly Arg Thr Ile Ser Lys
        355                 360                 365

Asp Leu Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Thr
    370                 375                 380

Thr Ala Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn
385                 390                 395                 400

Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser
                405                 410                 415

Cys Val Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Gly Pro Gln
            420                 425                 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
        435                 440                 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
    450                 455                 460

Asn Phe Met Pro Ile
465


<210> SEQ ID NO 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H3N2 NA FWD primer

<400> SEQUENCE: 13

gggaccacgc tgaacaataa                                                20


<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H3N2 NA REV primer

<400> SEQUENCE: 14
```

tgaaacggaa cacccaactc 20

<210> SEQ ID NO 15
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H3N8 NA FWD primer

<400> SEQUENCE: 15

gttcgccctc agaatgtaga a 21

<210> SEQ ID NO 16
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: H3N8 NA REV primer

<400> SEQUENCE: 16

cctatacgga cttcgatcct ttatt 25

<210> SEQ ID NO 17
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.390R

<400> SEQUENCE: 17

gaactccaat gtgcctgatg 20

<210> SEQ ID NO 18
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.259F

<400> SEQUENCE: 18

ctgcacacta atagatgccc ta 22

<210> SEQ ID NO 19
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.743F

<400> SEQUENCE: 19

ccaatctggc agaataagcg 20

<210> SEQ ID NO 20
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.1276F

<400> SEQUENCE: 20

gaagggagga ttcaagacct t 21

<210> SEQ ID NO 21
<211> LENGTH: 20
<212> TYPE: DNA

<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.1569F

<400> SEQUENCE: 21

ggttccagat caaaggtgtt 20

<210> SEQ ID NO 22
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.871R

<400> SEQUENCE: 22

cattcggaaa tgcaggtgtc a 21

<210> SEQ ID NO 23
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.HA.1408R

<400> SEQUENCE: 23

tcagcatttt ccctcaattg c 21

<210> SEQ ID NO 24
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.NA.429F

<400> SEQUENCE: 24

gggaccacgc tgaacaataa 20

<210> SEQ ID NO 25
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.NA.484R

<400> SEQUENCE: 25

tgaaacggaa cacccaactc 20

<210> SEQ ID NO 26
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.NA.975F

<400> SEQUENCE: 26

tcaggacttg ttggcgatac 20

<210> SEQ ID NO 27
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.NA.1023R

<400> SEQUENCE: 27

tcctggattc cctctctcat ta 22

<210> SEQ ID NO 28
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.1229F

<400> SEQUENCE: 28

actggtctgg ttattctggt attt 24

<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Ca.H3N2.1279R

<400> SEQUENCE: 29

tcttgtggcc ctcctcttat 20

<210> SEQ ID NO 30
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding HA protein of H3N8 CIV strain A/Ca/CT/85863/11

<400> SEQUENCE: 30

atgaaaacaa ccattatttt aatactactg acccattggg cctacagtca aaacccaatc 60

agtggcaata acacagccac actgtgtctg ggacaccatg cagtagcaaa tggaacattg 120

gtaaagacaa tgagtgatga tcaaattgag gtgacaaatg ctacagaatt agttcagagc 180

atttcaatgg ggaaaatatg caacaaatca tatagagttc tagatggaag aaattgcaca 240

ttaatagatg caatgctagg agacccccag tgtgacgcct ttcagtatga gagttgggac 300

ctctttatag aaagaagcaa tgctttcagc aattgctacc catatgacat ccctgactat 360

gcatcgctcc gatccattgt agcatcctca ggaacagtgg aattcacagt agagggattc 420

acatggacag gtgtcactca aaacggaaga agtggagcct gcaaaagggg atcagccgat 480

agtttcttta gccgactgaa ttggctaaca aaatctggaa gctcttaccc cacattgaat 540

gtgacaatgc ctaacaataa aaatttcgac aagctataca tctgggggat tcatcacccg 600

agctcgaatc aagagcagac aaaattgtac atccaagaat caggacgagt aacagtctca 660

acaaaaagaa gtcaacaaac aataatccct cacatcggat ctagaccgtt gatcagaggt 720

caatcaggca ggataagcat atactggacc attgtaaaac ctggagatat cctaatgata 780

aacagtaatg gcaacttagt tgcaccgcgg ggatatttca aattgaaccc aggaaaaagc 840

tctgtaatga gatccgatgt acccatagac atttgtgtgt ctgaatgtat tacaccaaat 900

ggaagcatct ccaacgacaa gccattccaa aatgtgaaca aagttacata tggaaaatgc 960

cccaagtata tcaggcaaaa cactttaaag ttggccactg ggatgaggaa tgtgccagaa 1020

aagcaaacca gaggaatctt tggggcgata gcgggattca tcgaaaacgg ctgggaagga 1080

atggttgatg ggtggtatgg gttccgatat caaaactctg aaggaacagg gcaagctgca 1140

gatctaaaga gcactcaagc agccatcgac cagattaatg gaaagttaaa cagggtgatt 1200

gaaagaacca atgagaaatt ccatcaaata gagaaggaat tctcagaagt agaagaaaga 1260

attcaggact tggagaaata tgtagaagac accaaaatag acctatggtc ctacaatgca 1320

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gaactgctgg tggctctaga aaatcaacat acaattgact taacagatgc agaaatgaat   1380

aaattatttg agaagactag acgccagtta agagaaaatg cagaagacat gggagatgga   1440

tgtttcaaga tttaccacaa gtgtgataat gcatgcattg aatcaataag aactggaaca   1500

tatgaccatt acatatacag agatgaagca ataaacaacc gatttcagat caaaggtgta   1560

gaattgaaat caggctacaa agattggata ctgtggattt cattcgccat atcatgcttc   1620

ttaatttgcg ttgttctatt gggtttcatt atgtggcctt gccaaaaagg caacatcaga   1680

tgcaacattt gcatt                                                    1695


<210> SEQ ID NO 31
<211> LENGTH: 565
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: HA protein of H3N8 CIV strain A/Ca/CT/85863/11

<400> SEQUENCE: 31

Met Lys Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Ala Tyr Ser
1               5                   10                  15

Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His
            20                  25                  30

His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Met Ser Asp Asp Gln
        35                  40                  45

Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ile Ser Met Gly
    50                  55                  60

Lys Ile Cys Asn Lys Ser Tyr Arg Val Leu Asp Gly Arg Asn Cys Thr
65                  70                  75                  80

Leu Ile Asp Ala Met Leu Gly Asp Pro Gln Cys Asp Ala Phe Gln Tyr
                85                  90                  95

Glu Ser Trp Asp Leu Phe Ile Glu Arg Ser Asn Ala Phe Ser Asn Cys
            100                 105                 110

Tyr Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala
        115                 120                 125

Ser Ser Gly Thr Val Glu Phe Thr Val Glu Gly Phe Thr Trp Thr Gly
    130                 135                 140

Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser Ala Asp
145                 150                 155                 160

Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Ser Ser Tyr
                165                 170                 175

Pro Thr Leu Asn Val Thr Met Pro Asn Asn Lys Asn Phe Asp Lys Leu
            180                 185                 190

Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Gln Glu Gln Thr Lys
        195                 200                 205

Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Lys Arg Ser
    210                 215                 220

Gln Gln Thr Ile Ile Pro His Ile Gly Ser Arg Pro Leu Ile Arg Gly
225                 230                 235                 240

Gln Ser Gly Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly Asp
                245                 250                 255

Ile Leu Met Ile Asn Ser Asn Gly Asn Leu Val Ala Pro Arg Gly Tyr
            260                 265                 270

Phe Lys Leu Asn Pro Gly Lys Ser Ser Val Met Arg Ser Asp Val Pro
        275                 280                 285
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Ile Asp Ile Cys Val Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Ser
    290                 295                 300

Asn Asp Lys Pro Phe Gln Asn Val Asn Lys Val Thr Tyr Gly Lys Cys
305                 310                 315                 320

Pro Lys Tyr Ile Arg Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg
                325                 330                 335

Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala Gly
            340                 345                 350

Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe
        355                 360                 365

Arg Tyr Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys Ser
    370                 375                 380

Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val Ile
385                 390                 395                 400

Glu Arg Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser Glu
                405                 410                 415

Val Glu Glu Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr Lys
            420                 425                 430

Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu Asn
        435                 440                 445

Gln His Thr Ile Asp Leu Thr Asp Ala Glu Met Asn Lys Leu Phe Glu
    450                 455                 460

Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asp Gly
465                 470                 475                 480

Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Glu Ser Ile
                485                 490                 495

Arg Thr Gly Thr Tyr Asp His Tyr Ile Tyr Arg Asp Glu Ala Ile Asn
            500                 505                 510

Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys Asp
        515                 520                 525

Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Ile Cys Val
    530                 535                 540

Val Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile Arg
545                 550                 555                 560

Cys Asn Ile Cys Ile
                565


<210> SEQ ID NO 32
<211> LENGTH: 1410
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein of H3N8 CIV strain
      A/Ca/CT/85863/11

<400> SEQUENCE: 32

atgaacccaa atcaaaagat aatagcaatt ggatctgcat cattggggat attaatcatt      60

aatatcattc tccatgtagt cagcattata gtaacagtac tggtcctcaa taacaataga     120

acagatctga actgcaaagg gacgatcata agagagtaca atgaaacagt aagagtagaa     180

aaacttactc aatggtacaa catcagtaca accaagtaca tagagagacc ttcaaatgaa     240

tattacatga acaacactga accactttgt gaggcccaag gctttgcacc attttccaaa     300

gataatggaa tacgaattgg gtcgagaggc catgtttttg tgataagaga accttttgta     360

tcatgttcac cctcagaatg tagaaccttt ttcctcacac agggctcatt actcaatgac     420
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aaacattcta acggcacaat aaaggatcga agtccgtata ggactctgat gagtgtcaaa    480

atagggcaat cacctaatgt atatcaagct aaatttgaat cggtggcatg gtcagcaaca    540

gcatgccatg atggaaaaaa atggatgaca gttggagtca cagggcccga caatcaagca    600

attgcagtag tgaactatgg aggtgttccg gttgatatta ttaattcatg ggcaggggat    660

attttaagaa cccaagaatc atcatgcacc tgcattaaag gagactgtta ttgggtaatg    720

actgatggac cggcaaatag gcaagctaat tataggatat tcaaagcaaa agatggaaga    780

gtaattggac aaactgatat aagtttcaat gggggacaca tagaggagtg ttcttgttac    840

cccaatgaag ggaaggtgga atgcatatgc agagacaatt ggactggaac aaacagacca    900

attctggtaa tatcttctga tctatcgtac acagttggat atttgtgtgc tggcattccc    960

actgacaccc ctaggggaga ggatagtcaa ttcacaggct catgtacaag tcctttggga   1020

aataaaggat acggtgtcaa aggtttcggg tttcgacaag gaactgacgt atgggccgga   1080

aggacaatta gtaggacttc aagatcagga ttcgaaataa taaaaatcag gaatggctgg   1140

acacagaata gtaaggacca aatcaggagg caagtgatta tcgatgacct aaattggtca   1200

ggatatagcg gttctttcac attgccggtt gaattaacaa aaaaaggatg tttggtcccc   1260

tgtttctggg ttgaaatgat tagaggtaaa cctgaagaaa caacaatatg gacctctagc   1320

agctccattg tgatgtgtgg agtagatcat aaaattgcca gttggtcatg gcacgatgga   1380

gcaattcttc cctttgacat cgataagatg                                    1410


<210> SEQ ID NO 33
<211> LENGTH: 470
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein of H3N8 CIV strain A/Ca/CT/85863/11

<400> SEQUENCE: 33

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Ser Ala Ser Leu Gly
1               5                   10                  15

Ile Leu Ile Ile Asn Ile Ile Leu His Val Val Ser Ile Ile Val Thr
            20                  25                  30

Val Leu Val Leu Asn Asn Asn Arg Thr Asp Leu Asn Cys Lys Gly Thr
        35                  40                  45

Ile Ile Arg Glu Tyr Asn Glu Thr Val Arg Val Glu Lys Leu Thr Gln
    50                  55                  60

Trp Tyr Asn Ile Ser Thr Thr Lys Tyr Ile Glu Arg Pro Ser Asn Glu
65                  70                  75                  80

Tyr Tyr Met Asn Asn Thr Glu Pro Leu Cys Glu Ala Gln Gly Phe Ala
                85                  90                  95

Pro Phe Ser Lys Asp Asn Gly Ile Arg Ile Gly Ser Arg Gly His Val
            100                 105                 110

Phe Val Ile Arg Glu Pro Phe Val Ser Cys Ser Pro Ser Glu Cys Arg
        115                 120                 125

Thr Phe Phe Leu Thr Gln Gly Ser Leu Leu Asn Asp Lys His Ser Asn
    130                 135                 140

Gly Thr Ile Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser Val Lys
145                 150                 155                 160

Ile Gly Gln Ser Pro Asn Val Tyr Gln Ala Lys Phe Glu Ser Val Ala
                165                 170                 175

Trp Ser Ala Thr Ala Cys His Asp Gly Lys Lys Trp Met Thr Val Gly
            180                 185                 190
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Val Thr Gly Pro Asp Asn Gln Ala Ile Ala Val Val Asn Tyr Gly Gly
        195                 200                 205

Val Pro Val Asp Ile Ile Asn Ser Trp Ala Gly Asp Ile Leu Arg Thr
    210                 215                 220

Gln Glu Ser Ser Cys Thr Cys Ile Lys Gly Asp Cys Tyr Trp Val Met
225                 230                 235                 240

Thr Asp Gly Pro Ala Asn Arg Gln Ala Asn Tyr Arg Ile Phe Lys Ala
                245                 250                 255

Lys Asp Gly Arg Val Ile Gly Gln Thr Asp Ile Ser Phe Asn Gly Gly
            260                 265                 270

His Ile Glu Glu Cys Ser Cys Tyr Pro Asn Glu Gly Lys Val Glu Cys
        275                 280                 285

Ile Cys Arg Asp Asn Trp Thr Gly Thr Asn Arg Pro Ile Leu Val Ile
    290                 295                 300

Ser Ser Asp Leu Ser Tyr Thr Val Gly Tyr Leu Cys Ala Gly Ile Pro
305                 310                 315                 320

Thr Asp Thr Pro Arg Gly Glu Asp Ser Gln Phe Thr Gly Ser Cys Thr
                325                 330                 335

Ser Pro Leu Gly Asn Lys Gly Tyr Gly Val Lys Gly Phe Gly Phe Arg
            340                 345                 350

Gln Gly Thr Asp Val Trp Ala Gly Arg Thr Ile Ser Arg Thr Ser Arg
        355                 360                 365

Ser Gly Phe Glu Ile Ile Lys Ile Arg Asn Gly Trp Thr Gln Asn Ser
    370                 375                 380

Lys Asp Gln Ile Arg Arg Gln Val Ile Ile Asp Asp Leu Asn Trp Ser
385                 390                 395                 400

Gly Tyr Ser Gly Ser Phe Thr Leu Pro Val Glu Leu Thr Lys Lys Gly
                405                 410                 415

Cys Leu Val Pro Cys Phe Trp Val Glu Met Ile Arg Gly Lys Pro Glu
            420                 425                 430

Glu Thr Thr Ile Trp Thr Ser Ser Ser Ser Ile Val Met Cys Gly Val
        435                 440                 445

Asp His Lys Ile Ala Ser Trp Ser Trp His Asp Gly Ala Ile Leu Pro
    450                 455                 460

Phe Asp Ile Asp Lys Met
465                 470


<210> SEQ ID NO 34
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding HA protein of H3N8 CIV strain
      A/canine/NY/120106.2/2011

<400> SEQUENCE: 34

atgaaaacaa ccattatttt aatactactg acccattggg cctacagtca aaacccaatc      60

agtggcaata acacagccac actgtgtctg ggacaccatg cagtagcaaa tggaacattg     120

gtaaagacaa tgagtgatga tcaaattgag gtgacaaatg ttacagaatt agttcagagc     180

atttcaatgg ggaaaatatg caacaaatca tatagagttc tagatggaag aaattgcaca     240

ttaatagatg caatgctagg agacccccag tgtgacgcct ttcagtatga gagttgggac     300

ctctttatag aaagaagcaa cgctttcagc aattgctacc catatgacat ccctgactat     360

gcatcgctcc gatccattgt agcatcctca ggaacagtgg aattcacagc agagggattc     420
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acatggacag gtgtcactca aaacggaaga agtggagcct gcaaaagggg atcagccgat    480

agtttcttta gccgactgaa ttggctaaca aaatctggaa gctcttaccc cacattgaat    540

gtaacaatgc ctaacaatga aaatttcgac aagctataca tctgggggat tcatcacccg    600

agctcgaatc aagagcagac aaaattgtac attcaagaat caggacgagt aacagtctca    660

acaaaaagaa gtcaacaaac aataattcct cacatcggat ctagaccgtt gatcagaggt    720

caatcaggca ggataagcat atactggacc attgtaaaac ctggagatat cctaatgata    780

aacagtaatg gcaacttagt tgcaccgcgg ggatatttca aattgaaccc aggaaaaagc    840

tctgtaatta gatccgatgt acccatagac atttgtgtgt ctgaatgtat tacaccaaat    900

ggaagcatct ccaacgacaa gccattccaa aatgtgaaca aagttacata tggaaaatgc    960

cccaagtata tcaggcaaaa cactttaaag ttggccactg ggatgaggaa tgtgccagaa   1020

aagcaaacca gaggaatctt tggggcgata gcgggattca tcgaaaacgg ctgggaagga   1080

atggttgatg ggtggtatgg gttccgatat caaaactctg aaggaacagg gcaagctgca   1140

gatctaaaga gcactcaagc agccatcgac cagattaatg gaaagttaaa cagggtgatt   1200

gaaagaacca atgagaaatt ccatcaaata gagaaggaat tctcagaagt agaaggaaga   1260

attcaggact tggagaaata tgtagaagac accaaaatag acctatggtc ctacaatgca   1320

gaactactgg tggctctaga aaatcaacat acaattgact taacagatgc agaaatgaat   1380

aaattatttg agaagactag acgccagtta agagaaaatg cagaagacat gggaaatgga   1440

tgtttcaaga tttaccacaa gtgtgataat gcatgcattg agtcaataag aactggaaca   1500

tatgaccatt acatatacag agatgaagca ataaacaacc gatttcagat caaaggtgta   1560

gaattgaaat caggctacaa agattggata ctgtggattt cattcgccat atcatgcttc   1620

ttaatttgcg ttgttctatt gggtttcatt atgtgggctt gccaaaaagg caacatcaga   1680

tgcaacattt gcatt                                                     1695


<210> SEQ ID NO 35
<211> LENGTH: 565
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: HA protein of H3N8 CIV strain
      A/canine/NY/120106.2/2011

<400> SEQUENCE: 35

Met Lys Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Ala Tyr Ser
1               5                   10                  15

Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His
            20                  25                  30

His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Met Ser Asp Asp Gln
        35                  40                  45

Ile Glu Val Thr Asn Val Thr Glu Leu Val Gln Ser Ile Ser Met Gly
    50                  55                  60

Lys Ile Cys Asn Lys Ser Tyr Arg Val Leu Asp Gly Arg Asn Cys Thr
65                  70                  75                  80

Leu Ile Asp Ala Met Leu Gly Asp Pro Gln Cys Asp Ala Phe Gln Tyr
                85                  90                  95

Glu Ser Trp Asp Leu Phe Ile Glu Arg Ser Asn Ala Phe Ser Asn Cys
            100                 105                 110

Tyr Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala
        115                 120                 125
```

```
Ser Ser Gly Thr Val Glu Phe Thr Ala Glu Gly Phe Thr Trp Thr Gly
    130                 135                 140

Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser Ala Asp
145                 150                 155                 160

Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Ser Ser Tyr
                165                 170                 175

Pro Thr Leu Asn Val Thr Met Pro Asn Asn Glu Asn Phe Asp Lys Leu
            180                 185                 190

Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Gln Glu Gln Thr Lys
        195                 200                 205

Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Lys Arg Ser
    210                 215                 220

Gln Gln Thr Ile Ile Pro His Ile Gly Ser Arg Pro Leu Ile Arg Gly
225                 230                 235                 240

Gln Ser Gly Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly Asp
                245                 250                 255

Ile Leu Met Ile Asn Ser Asn Gly Asn Leu Val Ala Pro Arg Gly Tyr
            260                 265                 270

Phe Lys Leu Asn Pro Gly Lys Ser Ser Val Ile Arg Ser Asp Val Pro
        275                 280                 285

Ile Asp Ile Cys Val Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Ser
    290                 295                 300

Asn Asp Lys Pro Phe Gln Asn Val Asn Lys Val Thr Tyr Gly Lys Cys
305                 310                 315                 320

Pro Lys Tyr Ile Arg Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg
                325                 330                 335

Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala Gly
            340                 345                 350

Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe
        355                 360                 365

Arg Tyr Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys Ser
    370                 375                 380

Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val Ile
385                 390                 395                 400

Glu Arg Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser Glu
                405                 410                 415

Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr Lys
            420                 425                 430

Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu Asn
        435                 440                 445

Gln His Thr Ile Asp Leu Thr Asp Ala Glu Met Asn Lys Leu Phe Glu
    450                 455                 460

Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn Gly
465                 470                 475                 480

Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Glu Ser Ile
                485                 490                 495

Arg Thr Gly Thr Tyr Asp His Tyr Ile Tyr Arg Asp Glu Ala Ile Asn
            500                 505                 510

Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys Asp
        515                 520                 525

Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Ile Cys Val
    530                 535                 540
```

```
Val Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile Arg
545                 550                 555                 560

Cys Asn Ile Cys Ile
                565


<210> SEQ ID NO 36
<211> LENGTH: 1410
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein of H3N8 CIV strain
      A/canine/NY/120106.2/2011

<400> SEQUENCE: 36

atgaatccaa atcaaaagat aatagcaatt ggatctgcat cattggggat attaatcatt      60

aatatcattc tccatgtagt cagcattata gtaacagtac tggtcctcaa taacaataga     120

acagatctga actgcaaagg gacgatcata agagagtaca atgaaacagt aagagtagaa     180

aaacttactc aatggtataa tatcagtaca attaagtaca tagagagacc ttcaaatgaa     240

tattacatga acaacactga accactttgt gaggcccaag gctttgcacc attttccaaa     300

gataatggaa tacgaattgg gtcgagaggc catgtttttg tgataagaga accttttgta     360

tcatgttcac cctcagaatg tagaaccttt ttcctcacac agggctcatt actcaatgac     420

aaacattcta acggcacaat aaaggatcga agtccgtata ggactctgat gagtgtcaaa     480

atagggcaat cacctaatgt atatcaagct aaatttgaat cggtggcatg gtcagcaaca     540

gcatgccatg atggaaaaaa atggatgaca gttggagtca cagggcccga caatcaagca     600

attgcagtag tgaactatgg aggtgttccg gttgatatta ttaattcatg ggcaggggat     660

attttaagaa cccaagaatc atcatgcacc tgcattaaag gagactgtta ttgggtaatg     720

actgatggac cggcaaatag gcaagctaat tataggatat tcaaagcaaa agatggaaga     780

gtaattggac aaactgatat aagtttcaat gggggacaca tagaggagtg ttcttgttac     840

cccaatgaag ggaaggtgga atgcatatgc agagacaatt ggactggaac aaacagacca     900

attctggtaa tatcttctga tctatcgtac acagttggat atttgtgtgc tggcattccc     960

actgacaccc ctaggggaga ggatagtcaa ttcacaggct catgtacaag tcctttggga    1020

aataaaggat acggtgtcaa aggtttcggg tttcgacaag gaactgacgt atgggccgga    1080

aggacaatta gtaggacttc aagatcagga ttcgaaataa taaaaatcag gaatggttgg    1140

acacagaata gtaaggacca aatcaggagg caagtgatta tcgatgaccc aaattggtca    1200

ggatatagcg gttctttcac attgccggtt gaattaacaa aaaaaggatg tttggtcccc    1260

tgtttctggg ttgaaatgat tagaggtaaa cctgaagaaa caacaatatg gacctctagc    1320

agctccattg tgatgtgtgg agtagatcat aaaattgcca gttggtcatg gcacgatgga    1380

gcaattcttc cctttgacat cgataagatg                                     1410


<210> SEQ ID NO 37
<211> LENGTH: 470
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein of H3N8 CIV strain
      A/canine/NY/120106.2/2011

<400> SEQUENCE: 37

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Ser Ala Ser Leu Gly
1               5                   10                  15
```

```
Ile Leu Ile Ile Asn Ile Ile Leu His Val Val Ser Ile Ile Val Thr
            20                  25                  30

Val Leu Val Leu Asn Asn Asn Arg Thr Asp Leu Asn Cys Lys Gly Thr
        35                  40                  45

Ile Ile Arg Glu Tyr Asn Glu Thr Val Arg Val Glu Lys Leu Thr Gln
    50                  55                  60

Trp Tyr Asn Ile Ser Thr Ile Lys Tyr Ile Glu Arg Pro Ser Asn Glu
65                  70                  75                  80

Tyr Tyr Met Asn Asn Thr Glu Pro Leu Cys Glu Ala Gln Gly Phe Ala
                85                  90                  95

Pro Phe Ser Lys Asp Asn Gly Ile Arg Ile Gly Ser Arg Gly His Val
            100                 105                 110

Phe Val Ile Arg Glu Pro Phe Val Ser Cys Ser Pro Ser Glu Cys Arg
        115                 120                 125

Thr Phe Phe Leu Thr Gln Gly Ser Leu Leu Asn Asp Lys His Ser Asn
    130                 135                 140

Gly Thr Ile Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser Val Lys
145                 150                 155                 160

Ile Gly Gln Ser Pro Asn Val Tyr Gln Ala Lys Phe Glu Ser Val Ala
                165                 170                 175

Trp Ser Ala Thr Ala Cys His Asp Gly Lys Lys Trp Met Thr Val Gly
            180                 185                 190

Val Thr Gly Pro Asp Asn Gln Ala Ile Ala Val Val Asn Tyr Gly Gly
        195                 200                 205

Val Pro Val Asp Ile Ile Asn Ser Trp Ala Gly Asp Ile Leu Arg Thr
    210                 215                 220

Gln Glu Ser Ser Cys Thr Cys Ile Lys Gly Asp Cys Tyr Trp Val Met
225                 230                 235                 240

Thr Asp Gly Pro Ala Asn Arg Gln Ala Asn Tyr Arg Ile Phe Lys Ala
                245                 250                 255

Lys Asp Gly Arg Val Ile Gly Gln Thr Asp Ile Ser Phe Asn Gly Gly
            260                 265                 270

His Ile Glu Glu Cys Ser Cys Tyr Pro Asn Glu Gly Lys Val Glu Cys
        275                 280                 285

Ile Cys Arg Asp Asn Trp Thr Gly Thr Asn Arg Pro Ile Leu Val Ile
    290                 295                 300

Ser Ser Asp Leu Ser Tyr Thr Val Gly Tyr Leu Cys Ala Gly Ile Pro
305                 310                 315                 320

Thr Asp Thr Pro Arg Gly Glu Asp Ser Gln Phe Thr Gly Ser Cys Thr
                325                 330                 335

Ser Pro Leu Gly Asn Lys Gly Tyr Gly Val Lys Gly Phe Gly Phe Arg
            340                 345                 350

Gln Gly Thr Asp Val Trp Ala Gly Arg Thr Ile Ser Arg Thr Ser Arg
        355                 360                 365

Ser Gly Phe Glu Ile Ile Lys Ile Arg Asn Gly Trp Thr Gln Asn Ser
    370                 375                 380

Lys Asp Gln Ile Arg Arg Gln Val Ile Ile Asp Asp Pro Asn Trp Ser
385                 390                 395                 400

Gly Tyr Ser Gly Ser Phe Thr Leu Pro Val Glu Leu Thr Lys Lys Gly
                405                 410                 415

Cys Leu Val Pro Cys Phe Trp Val Glu Met Ile Arg Gly Lys Pro Glu
            420                 425                 430

Glu Thr Thr Ile Trp Thr Ser Ser Ser Ser Ile Val Met Cys Gly Val
```

435 440 445

Asp His Lys Ile Ala Ser Trp Ser Trp His Asp Gly Ala Ile Leu Pro
450 455 460

Phe Asp Ile Asp Lys Met
465 470

<210> SEQ ID NO 38
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding HA protein of H3N8 CIV strain WY/86033/07

<400> SEQUENCE: 38

```
atgaagacaa ccattatttt aatactactg acccattggg cccacagtca aaacccaatc     60

agtggcaata acacagccac actgtgtctg ggacaccatg cagtagcaaa tggaacatta    120

gtaaaaacaa tgagtgatga tcaaattgag gtgacaaatg ctacagaatt agttcagagc    180

atttcaatgg ggaaaatatg caacaaatca tatagaattc tagatggaag aaattgcaca    240

ttaatagatg caatgctagg agacccccac tgtgacgcct ttcagtatga gagttgggac    300

ctctttatag aaagaagcaa cgctttcagc aattgctacc catatgacat ccctgactat    360

gcatcgctcc gatccattgt agcatcctca ggaacagtga aattcacagc agagggattc    420

acatggacag gtgtcactca aaacggaaga agtggagcct gcaaaagggg atcagccgat    480

agtttcttta gccgactgaa ttggctaaca aaatctggaa gctcttaccc cacattgaat    540

gtgacaatgc ctaacaataa aaatttcgac aagctataca tctgggggat tcatcaccca    600

agctcaaatc aagagcagac aaaattgtac atccaagaat caggacgagt aacagtctca    660

acaaaaagaa gtcaacaaac aataatccct aacatcggat ctagaccgtt ggtcagaggt    720

caatcaggca ggataagcat atactggacc attgtaaaac ctggagatat cctaatgata    780

aacagtaatg gcaacttagt tgcaccgcgg ggatatttta aactgaacac agggaaaagc    840

tctgtaatga gatccgatgt acccatagac atttgtgtgt ctgaatgtat tacaccaaat    900

ggaagcatct ccaacgacaa gccattccaa aatgtgaaca aagttacata tggaaaatgc    960

cccaagtata tcaggcaaaa cactttaaag ctggccactg ggatgaggaa tgtaccagaa   1020

aagcaaacca gaggaatctt tggagcaata gcgggattca tcgaaaacgg ctgggaagga   1080

atggttgatg ggtggtatgg gttccgatat caaaactctg aaggaacagg gcaagctgca   1140

gatctaaaga gcactcaagc agccatcgac cagattaatg gaaagttaaa cagagtgatt   1200

gaaagaacca atgagaaatt ccatcaaata gagaaggaat tctcagaagt agaaggaaga   1260

attcaggact tggagaaata tgtagaagac accaaaatag acctatggtc ctacaatgca   1320

gaattgctgg tggctctaga aaatcaacat acaattgact taacagatgc aaaaatgaat   1380

aaattatttg agaagactag acgccagttg agagaaaacg cagaagacat gggaggtgga   1440

tgtttcaaga tttatcacaa atgtgataat gcatgcattg aatcaataag aactggaaca   1500

tatgaccatt acatatacag agatgaagca ttaaacaacc gatttcagat caaaggtgta   1560

gagttgaaat caggctacaa agattggata ctgtggattt cattcgccat atcatgcttc   1620

ttaatttgcg ttgttctatt gggtttcatt atgtgggctt gccaaaaagg caacatcaga   1680

tgcaacattt gcatt                                                    1695
```

<210> SEQ ID NO 39

<211> LENGTH: 565
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: HA protein of H3N8 CIV strain WY/86033/07

<400> SEQUENCE: 39

```
Met Lys Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Ala His Ser
1               5                   10                  15

Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His
            20                  25                  30

His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Met Ser Asp Asp Gln
        35                  40                  45

Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ile Ser Met Gly
    50                  55                  60

Lys Ile Cys Asn Lys Ser Tyr Arg Ile Leu Asp Gly Arg Asn Cys Thr
65                  70                  75                  80

Leu Ile Asp Ala Met Leu Gly Asp Pro His Cys Asp Ala Phe Gln Tyr
                85                  90                  95

Glu Ser Trp Asp Leu Phe Ile Glu Arg Ser Asn Ala Phe Ser Asn Cys
            100                 105                 110

Tyr Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala
        115                 120                 125

Ser Ser Gly Thr Val Lys Phe Thr Ala Glu Gly Phe Thr Trp Thr Gly
    130                 135                 140

Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser Ala Asp
145                 150                 155                 160

Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Ser Ser Tyr
                165                 170                 175

Pro Thr Leu Asn Val Thr Met Pro Asn Asn Lys Asn Phe Asp Lys Leu
            180                 185                 190

Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Gln Glu Gln Thr Lys
        195                 200                 205

Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Lys Arg Ser
    210                 215                 220

Gln Gln Thr Ile Ile Pro Asn Ile Gly Ser Arg Pro Leu Val Arg Gly
225                 230                 235                 240

Gln Ser Gly Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly Asp
                245                 250                 255

Ile Leu Met Ile Asn Ser Asn Gly Asn Leu Val Ala Pro Arg Gly Tyr
            260                 265                 270

Phe Lys Leu Asn Thr Gly Lys Ser Ser Val Met Arg Ser Asp Val Pro
        275                 280                 285

Ile Asp Ile Cys Val Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Ser
    290                 295                 300

Asn Asp Lys Pro Phe Gln Asn Val Asn Lys Val Thr Tyr Gly Lys Cys
305                 310                 315                 320

Pro Lys Tyr Ile Arg Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg
                325                 330                 335

Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala Gly
            340                 345                 350

Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe
        355                 360                 365

Arg Tyr Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys Ser
    370                 375                 380
```

-continued

```
Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val Ile
385                 390                 395                 400

Glu Arg Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser Glu
                405                 410                 415

Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr Lys
            420                 425                 430

Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu Asn
        435                 440                 445

Gln His Thr Ile Asp Leu Thr Asp Ala Lys Met Asn Lys Leu Phe Glu
    450                 455                 460

Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Gly Gly
465                 470                 475                 480

Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Glu Ser Ile
                485                 490                 495

Arg Thr Gly Thr Tyr Asp His Tyr Ile Tyr Arg Asp Glu Ala Leu Asn
            500                 505                 510

Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys Asp
        515                 520                 525

Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Ile Cys Val
    530                 535                 540

Val Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile Arg
545                 550                 555                 560

Cys Asn Ile Cys Ile
                565
```

<210> SEQ ID NO 40
<211> LENGTH: 1410
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein of H3N8 CIV strain WY/86033/07

<400> SEQUENCE: 40

```
atgaatccaa atcaaaagat aatagcaatt ggatttgcat cattggggat attaatcatt      60

aatgtcattc tccatgtagt cagcattata gtaacagtac tggtcctcaa taacaataga     120

acagatctga actacaaagg gacgatcata agagaataca atgaaacagt aagagtagaa     180

aaacttactc aatggtataa taccagtaca attaagtaca tagagagacc ttcaaatgaa     240

tactacatga ataacactga accactttgt gaggcccaag gctttgcacc attttccaaa     300

gataatggaa tacgaattgg gtcgagaggc catgtttttg tgataagaga accttttgta     360

tcatgttcgc cctcagaatg tagaaccttt ttcctcacac agggctcatt actcaatgac     420

aaacattcta acggcacaat aaaggatcga agtccgtata ggactttgat gagtgtcaaa     480

atagggcaat cacctaatgt atatcaagct agatttgaat cggtggcatg gtcagcaaca     540

gcatgccatg atggaaaaaa atggatgaca gttggagtca caggccccga caatcaagca     600

attgcagtag tgaactatgg aggtgttccg gttgatatta ttaattcatg ggcaggggat     660

attttaagaa cccaagagtc atcatgcacc tgcattaaag gagactgtta ttgggtaatg     720

actgatggac cggcaaatag gcaagctgaa tataggatat tcaaagcaaa agatggaaga     780

gtaattgggc aaactgatat aagtttcaat gggggacaca tagaggagtg ttcttgttac     840

cccaatgaag ggaaggtgga atgcatatgc agggacaatt ggactggaac aaatagacca     900

attctggtaa tatcttctga tctatcgtac acagttggat atttgtgtgc tggcattccc     960
```

```
actgacactc ctaggggaga ggatagtcaa ttcacaggct catgtacaag tcctttggga   1020

aataagggat acggtgtaaa aggcttcggg tttcgacaag gaactgacgt atgggccgga   1080

aggacaatta gtagaacttc aagatcagga ttcgaaataa taaaaatcag gaatggttgg   1140

acacagaaca gtaaggacca aatcaggagg caagtgatta tcgatgaccc aaattggtca   1200

ggatatagcg gttctttcac attgccggtt gaactgacaa aaaagggatg tttggtcccc   1260

tgtttctggg ttgaaatgat tagaggtaaa cctgaagaaa caacaatatg gacctctagc   1320

agctccattg tgatgtgtgg agtagatcat aaaattgcca gttggtcatg gcacgatgga   1380

gctattcttc cctttgacat cgataagatg                                    1410
```

```
<210> SEQ ID NO 41
<211> LENGTH: 470
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: NA protein of H3N8 CIV strain WY/86033/07

<400> SEQUENCE: 41

Met Asn Pro Asn Gln Lys Ile Ile Ala Ile Gly Phe Ala Ser Leu Gly
1               5                   10                  15

Ile Leu Ile Ile Asn Val Ile Leu His Val Val Ser Ile Ile Val Thr
            20                  25                  30

Val Leu Val Leu Asn Asn Asn Arg Thr Asp Leu Asn Tyr Lys Gly Thr
        35                  40                  45

Ile Ile Arg Glu Tyr Asn Glu Thr Val Arg Val Glu Lys Leu Thr Gln
    50                  55                  60

Trp Tyr Asn Thr Ser Thr Ile Lys Tyr Ile Glu Arg Pro Ser Asn Glu
65                  70                  75                  80

Tyr Tyr Met Asn Asn Thr Glu Pro Leu Cys Glu Ala Gln Gly Phe Ala
                85                  90                  95

Pro Phe Ser Lys Asp Asn Gly Ile Arg Ile Gly Ser Arg Gly His Val
            100                 105                 110

Phe Val Ile Arg Glu Pro Phe Val Ser Cys Ser Pro Ser Glu Cys Arg
        115                 120                 125

Thr Phe Phe Leu Thr Gln Gly Ser Leu Leu Asn Asp Lys His Ser Asn
    130                 135                 140

Gly Thr Ile Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser Val Lys
145                 150                 155                 160

Ile Gly Gln Ser Pro Asn Val Tyr Gln Ala Arg Phe Glu Ser Val Ala
                165                 170                 175

Trp Ser Ala Thr Ala Cys His Asp Gly Lys Lys Trp Met Thr Val Gly
            180                 185                 190

Val Thr Gly Pro Asp Asn Gln Ala Ile Ala Val Val Asn Tyr Gly Gly
        195                 200                 205

Val Pro Val Asp Ile Ile Asn Ser Trp Ala Gly Asp Ile Leu Arg Thr
    210                 215                 220

Gln Glu Ser Ser Cys Thr Cys Ile Lys Gly Asp Cys Tyr Trp Val Met
225                 230                 235                 240

Thr Asp Gly Pro Ala Asn Arg Gln Ala Glu Tyr Arg Ile Phe Lys Ala
                245                 250                 255

Lys Asp Gly Arg Val Ile Gly Gln Thr Asp Ile Ser Phe Asn Gly Gly
            260                 265                 270

His Ile Glu Glu Cys Ser Cys Tyr Pro Asn Glu Gly Lys Val Glu Cys
```

```
        275                 280                 285

Ile Cys Arg Asp Asn Trp Thr Gly Thr Asn Arg Pro Ile Leu Val Ile
    290                 295                 300

Ser Ser Asp Leu Ser Tyr Thr Val Gly Tyr Leu Cys Ala Gly Ile Pro
305                 310                 315                 320

Thr Asp Thr Pro Arg Gly Glu Asp Ser Gln Phe Thr Gly Ser Cys Thr
                325                 330                 335

Ser Pro Leu Gly Asn Lys Gly Tyr Gly Val Lys Gly Phe Gly Phe Arg
            340                 345                 350

Gln Gly Thr Asp Val Trp Ala Gly Arg Thr Ile Ser Arg Thr Ser Arg
        355                 360                 365

Ser Gly Phe Glu Ile Ile Lys Ile Arg Asn Gly Trp Thr Gln Asn Ser
    370                 375                 380

Lys Asp Gln Ile Arg Arg Gln Val Ile Ile Asp Asp Pro Asn Trp Ser
385                 390                 395                 400

Gly Tyr Ser Gly Ser Phe Thr Leu Pro Val Glu Leu Thr Lys Lys Gly
                405                 410                 415

Cys Leu Val Pro Cys Phe Trp Val Glu Met Ile Arg Gly Lys Pro Glu
            420                 425                 430

Glu Thr Thr Ile Trp Thr Ser Ser Ser Ser Ile Val Met Cys Gly Val
        435                 440                 445

Asp His Lys Ile Ala Ser Trp Ser Trp His Asp Gly Ala Ile Leu Pro
    450                 455                 460

Phe Asp Ile Asp Lys Met
465                 470
```

<210> SEQ ID NO 42
<211> LENGTH: 1410
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA encoding NA protein of H3N8 CIV strain A/canine/NY/120106

<400> SEQUENCE: 42

```
atgaacccaa atcaaaagat aatagcaatt ggatctgcat cattggggat attaatcatt     60

aatatcattc tccatgtagt cagcattata gtaacagtac tggtcctcaa taacaataga    120

acagatctga actgcaaagg gacgatcata agagagtaca atgaaacagt aagagtagaa    180

aaacttactc aatggtataa tatcagtaca attaagtaca tagagagacc ttcaaatgaa    240

tattacatga acaacactga accactttgt gaggcccaag gctttgcacc attttccaaa    300

gataatggaa tacgaattgg gtcgagaggc catgtttttg tgataagaga accttttgta    360

tcatgttcac cctcagaatg tagaaccttt ttcctcacac agggctcatt actcaatgac    420

aaacattcta acggcacaat aaaggatcga agtccgtata ggactctgat gagtgtcaaa    480

atagggcaat cacctaatgt atatcaagct aaatttgaat cggtggcatg gtcagcaaca    540

gcatgccatg atggaaaaaa atggatgaca gttggagtca cagggcccga caatcaagca    600

attgcagtag tgaactatgg aggtgttccg gttgatatta ttaattcatg ggcaggggat    660

attttaagaa cccaagaatc atcatgcacc tgcattaaag gagactgtta ttgggtaatg    720

actgatggac cggcaaatag gcaagctaat tataggatat tcaaagcaaa agatggaaga    780

gtaattggac aaactgatat aagtttcaat gggggacaca tagaggagtg ttcttgttac    840

cccaatgaag ggaaggtgga atgcatatgc agagacaatt ggactggaac aaacagacca    900
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-continued

attctggtaa tatcttctga tctatcgtac acagttggat atttgtgtgc tggcattccc	960
actgacaccc ctaggggaga ggatagtcaa ttcacaggct catgtacaag tcctttggga	1020
aataaaggat acggtgtcaa aggtttcggg tttcgacaag gaactgacgt atgggccgga	1080
aggacaatta gtaggacttc aagatcagga ttcgaaataa taaaaatcag gaatggttgg	1140
acacagaata gtaaggacca aatcaggagg caagtgatta tcgatgaccc aaattggtca	1200
ggatatagcg gttctttcac attgccggtt gaattaacaa aaaaaggatg tttggtcccc	1260
tgtttctggg ttgaaatgat tagaggtaaa cctgaagaaa caacaatatg gacctctagc	1320
agctccattg tgatgtgtgg agtagatcat aaaattgcca gttggtcatg gcacgatgga	1380
gcaattcttc cctttgacat cgataagatg	1410

What we claim is:

1. A composition comprising an H3N2 influenza virus derived from canine isolates (H3N2 CIV), wherein the H3N2 CIV comprises,
   a) a polynucleotide encoding an HA protein having at least 99.6% sequence identity to the sequence as set forth in SEQ ID NO:2, 4, or 6; and/or
   b) a polynucleotide encoding an NA protein having at least 99% sequence identity to the sequence as set forth in SEQ ID NO:8, 10 or 12; and/or
   c) an HA polynucleotide having at least 99.2% sequence identity to the sequence as set forth in SEQ ID NO:1; or having at least 99.6% sequence identity to the sequence as set forth in SEQ ID NO:3; or having at least 99.9% sequence identity to the sequence as set forth in SEQ ID NO:5; and/or
   d) an NA polynucleotide having at least 99.9% sequence identity to the sequence set forth in SEQ ID NO:7 or 9.

2. The composition of claim 1, wherein the H3N2 CIV is deposited at ATCC under the deposit number of PTA-122265 or PTA-122266 or is a progeny or descendant of PTA-122265 or PTA-122266 wherein the progeny or descendant comprises a polynucleotide encoding an HA protein having at least 99.6% sequence identity to the sequence as set forth in SEQ ID NO:2, 4, or 6.

3. The composition of claim 1, wherein the H3N2 CIV is deposited at ATCC under the deposit number of PTA-122265 or PTA-122266, or is a progeny or descendant of PTA-122265 or PTA-122266, wherein the progeny or descendant comprises a polynucleotide encoding an NA protein having at least 99% sequence identity to the sequence as set forth in SEQ ID NO:8, 10, or 12.

4. The composition of claim 1, wherein the H3N2 CIV is inactivated.

5. The composition of claim 1, wherein the composition further comprises an inactivated H3N8 CIV.

6. The composition of claim 5, wherein the H3N8 CIV comprises a polynucleotide encoding an HA protein having at least 98% sequence identity SEQ ID NO:31, 35, or 39.

7. The composition of claim 5, wherein the H3N8 CIV comprises polynucleotide encoding an NA protein having at least 98% sequence identity SEQ ID NO:33, 37, or 41.

8. The composition of claim 1, wherein the composition or vaccine further comprises one or more pharmaceutically or veterinarily acceptable carrier, adjuvant, vehicle, or excipient.

9. A method of vaccinating an animal, or for inducing an immunogenic or protective response against influenza virus infection in canines (CIV), or for reducing CIV viral shedding in an infected animal, comprising at least one administration of the composition of claim 1.

10. The method of claim 9, wherein the animal is a canine or feline.

11. A diagnostic method of the infection of H3N2 influenza virus in dogs or cats (H3N2 CIV), wherein a sample of physiological fluid or a dog or cat tissue sampling and a diagnostic reagent specific to H3N2 CIV are put together, and the potential presence of H3N2 CIV antigen, antibody or nucleic acid is revealed within this sample or sampling, wherein the diagnostic reagent specific to H3N2 CIV comprises
   a) a polynucleotide encoding an HA protein having at least 99.6% sequence identity to the sequence as set forth in SEQ ID NO:2, 4, or 6; and/or
   b) a polynucleotide encoding an NA protein having at least 99% sequence identity to the sequence as set forth in SEQ ID NO:8, 10 or 12; and/or
   c) an HA polynucleotide having at least 99.2% sequence identity to the sequence as set forth in SEQ ID NO:1; or having at least 99.6% sequence identity to the sequence as set forth in SEQ ID NO:3; or having at least 99.9% sequence identity to the sequence as set forth in SEQ ID NO:5; and/or
   d) an NA polynucleotide having at least 99.9% sequence identity to the sequence set forth in SEQ ID NO:7 or 9.

12. The diagnostic method of claim 11, wherein the diagnostic reagent comprises a specific H3N2 CIV antigen selected from the group consisting of HA and NA, and wherein the antigen allows the detection of antibodies within the sample or sampling as being H3N2 CIV.

13. The diagnostic method of claim 11, wherein the method is Western blotting, immunofluoroescence, ELISA or immunochromatography.

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